























Protein tyrosine phosphatase receptor type Q in cerebrospinal fluid reflects ependymal cell dysfunction and is a potential biomarker for adult chronic hydrocephalus

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Background and purpose: Protein tyrosine phosphatase receptor type Q (PTPRQ) was extracted from the cerebrospinal fluid (CSF) of patients with *probable* idiopathic normal-pressure hydrocephalus (iNPH) by proteome analysis. We aimed to assess the feasibility of using CSF PTPRQ concentrations for the additional diagnostic criterion of iNPH in Japanese and Finnish populations.

Methods: We compared PTPRQ concentrations among patients with *probable* iNPH and neurologically healthy individuals (normal control [NC] group), patients with normal-pressure hydrocephalus (NPH) of acquired and congenital/developmental aetiologies, patients with Alzheimer's disease and patients with Parkinson's disease in a Japanese analysis cohort. A corresponding iNPH group and NC group in a Finnish cohort was used for validation. Patients in the Finnish cohort who underwent biopsy were classified into two groups based on amyloid and/or tau deposition. We measured PTPRQ expression levels in autopsied brain specimens of iNPH patients and the NC group.

Results: Cerebrospinal fluid PTPRQ concentrations in the patients with NPH of idiopathic, acquired and congenital/developmental aetiologies were significantly higher than those in the NC group and those with Parkinson's disease, but iNPH showed no significant differences when compared with those in the Alzheimer's disease group. For the patients with iNPH, the area under the receiver-operating characteristic curve was 0.860 in the Japanese iNPH and 0.849 in the Finnish iNPH cohorts. Immunostaining and *in situ* hybridization revealed PTPRQ expression in the ependymal cells and choroid plexus. It is highly possible that the elevated PTPRQ levels in the CSF are related to ependymal dysfunction from ventricular expansion.

Conclusions: Cerebrospinal fluid PTPRQ levels indicated the validity of this assay for auxiliary diagnosis of adult chronic hydrocephalus.

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Introduction

Idiopathic normal-pressure hydrocephalus (iNPH) is prevalent among older adults and is characterized by

Hakim's triad, which consists of gait disturbances, cognitive impairment and urinary incontinence [1]. Although these symptoms are improved by cerebrospinal fluid (CSF) shunts, the aetiology and pathophysiology of iNPH remain enigmatic. However, iNPH is thought to stem from difficulties in the clearance of waste metabolites from the brain [2–4].

Diagnosis of iNPH is primarily based on symptoms and diagnostic imaging [5,6]; however, CSF-based assays are used as auxiliary diagnostic tools. For example, studies have measured the levels of CSF biomarkers for iNPH, such as amyloid β 1-42 (A β 42), tau phosphorylated at threonine 181 (p-tau), and total tau (t-tau), which are also associated with concomitant Alzheimer's disease (AD) [7,8]. In one study, these biomarkers predicted that CSF shunt placement would provide limited improvement of symptoms in patients with iNPH with concomitant AD [9]. Further, a variety of CSF protein biomarkers have been described [10–13], most of them being reduced in patients with iNPH compared with healthy individuals. Likely reasons for this reduction are delayed CSF clearance, an increase in the overall CSF volume due to iNPH [14], altered metabolism, and astrocyte activation [11,15]. Although previous studies have focused on AD-related proteins for the diagnosis of iNPH, most protein biomarkers were not directly associated with the underlying mechanisms of iNPH pathology. The lack of knowledge regarding the specific pathology of iNPH presents a challenge for identifying potential biomarkers for this disorder, therefore, it is necessary to discover new diagnostic biomarkers to help understand the pathophysiology of iNPH.

Recently, protein tyrosine phosphatase receptor type Q (PTPRQ) was extracted from the CSF of patients with iNPH by proteome analysis. PTPRQ has been reported to be involved in iNPH only once before [16]. The estimated molecular weight of PTPRQ in the CSF was approximately 170 kDa when the antibody for its extracellular domain was used [16]. The PTPRQ gene is involved in hearing loss (i.e., deafness autosomal recessive 84A [DFNB84A]) [17]. Nayak et al. [18] reported that the extracellular region of PTPRQ in the vestibular end organ is modified with dermatan sulphate, and, depending on the location of the dermatan sulphate on PTPRQ, neighbouring stereocilia adhere to or repel each other in order to maintain a proper distance between the cilia. However, the function of PTPRQ in the central nervous system is largely unknown. In the present study, we investigated the role of PTPRQ in *probable* iNPH and carried out the first intracerebral analysis of PTPRQ expression in autopsied brains of patients with iNPH.

Material and methods

Ethics statement

This study was reviewed and approved by the ethics committees of Juntendo University Hospital and Kuopio University Hospital (KUH) and conforms to the World Medical Association Declaration of Helsinki. All patients included in the study or their relatives gave informed consent for participation in the study. If the clinician suspected that dementia would significantly affect the capacity of the patient to consent, the next of kin or guardian consented on behalf of the patient. When consent was obtained from a participant's proxy, the patient's opinion was asked and considered, and no patients were recruited against their will.

Study design

In the first part of the study, we evaluated two cohorts of patients with probable iNPH: a Japanese cohort for analysis and a Finnish cohort for validation. We measured and compared the differences in the concentrations of CSF protein biomarkers for differential diagnosis among four groups: Japanese patients with probable iNPH (Japanese iNPH group), cognitively normal healthy individuals, hereafter referred to as the normal control (NC) group, patients with Alzheimer's disease (AD) and patients with Parkinson's disease (PD; Fig. 1). We validated the results for the NC group and patients with iNPH (Finnish iNPH group) in the Finnish cohort. Next, we intended to clarify the role and significance of PTPRQ in acquired (secondary) normal-pressure hydrocephalus (NPH; in which symptoms appeared subsequent to a preceding neurological injury, e.g., subarachnoid haemorrhage, meningitis) and congenital/developmental aetiologies, which present with pan-ventriculomegaly, wide foramen of Magendie and large cisterna magna [19].

In the second part of the study, immunostaining and mRNA expression analyses were performed in autopsied brain specimens of NCs and patients with iNPH.

Study population

This study included two cohorts: one from Japan and the other from Finland. Patients who had at least one component of the NPH triad (gait disturbance, dementia, and urinary incontinence), and those with ventriculomegaly on computed tomography (CT)/magnetic resonance imaging (MRI) were enrolled as

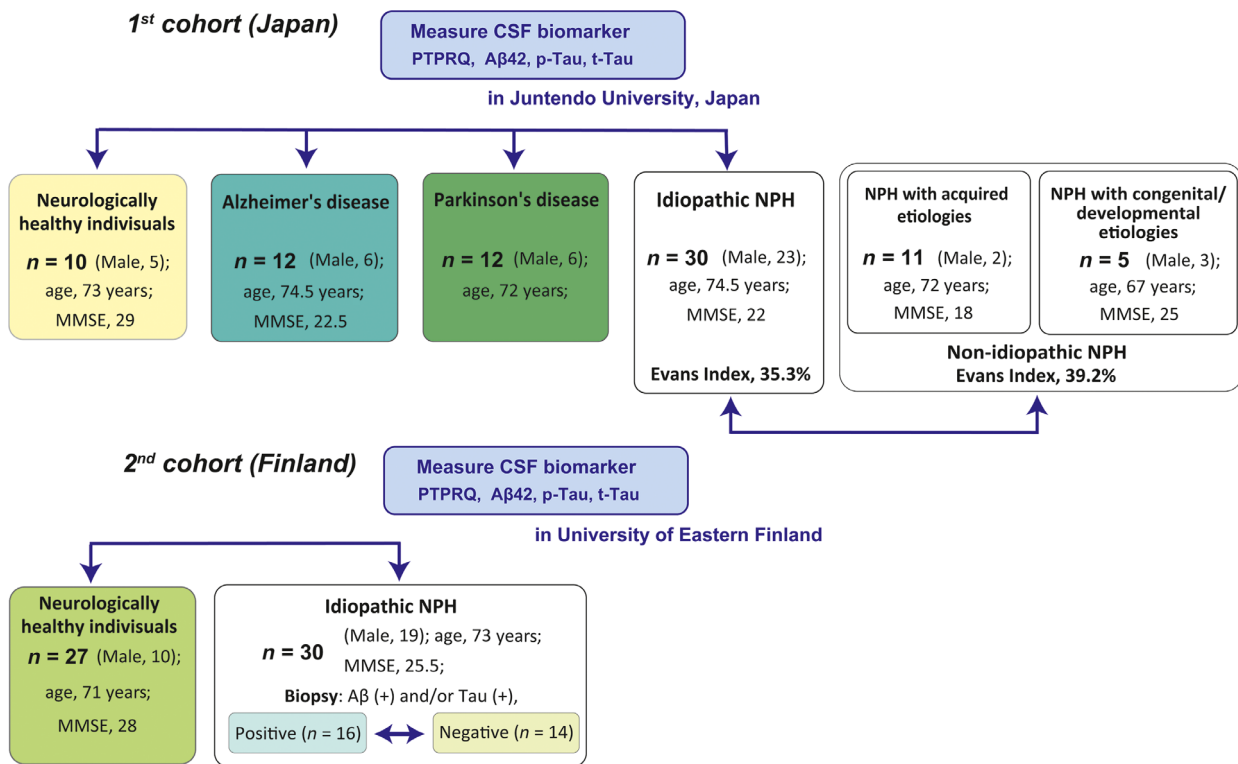


Figure 1 Study design. Aβ42, amyloid β 1-42; CSF, cerebrospinal fluid; NC, healthy controls (individuals with normal cognition); iNPH, idiopathic normal-pressure hydrocephalus; MMSE, Mini-Mental State Examination; PTPRQ, protein tyrosine phosphatase receptor type Q; PD, Parkinson's disease; p-tau, tau phosphorylated at threonine 181; t-tau, total tau. [Colour figure can be viewed at wileyonlinelibrary.com]

possible iNPH patients. In accordance with the Japanese guidelines for the management of iNPH, *probable* iNPH was defined as the presence of at least one component of the NPH triad with tight high convexity on CT/MRI or improvement of symptoms after CSF tap test or drainage [6]. CSF shunt surgeries were performed in patients with probable iNPH. A retrospective analysis of the detailed course of clinical symptoms was performed. Evaluation items included Mini Mental State Examination (MMSE) scores [19,20] and iNPH Grading Scale (iNPHGS) scores [21].

The first cohort in Japan comprised the Japanese NC group, which consisted of 10 subjects (median age 73 years; five men); these patients were aged ≥ 60 years and had no known brain diseases or subjective declines in cognition and MMSE scores ≥ 26 ; the Japanese iNPH group consisted of 30 consecutive patients with iNPH (median age 74.5 years; 23 men) who received a lumboperitoneal shunt intervention between February 2013 and April 2015; the AD group consisted of 12 patients with AD (median age 74.5 years; six men), diagnosed by a neurologist according to the National Institute of Neurological and Communicative

Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria for probable AD [22]; finally, the PD group consisted of 12 patients with PD (median age 72 years; six men), diagnosed by a neurologist according to the Movement Disorder Society Clinical Diagnostic Criteria for PD [23] (Table 1). An additional survey was conducted to compare iNPH with non-idiopathic NPH: 11 NPH patients with acquired aetiologies (median age 72 years; two men) and five NPH patients with congenital/developmental aetiologies (median age 67 years; three men; Table 2). The whole Japanese cohort was recruited from the Department of Neurology and Neurosurgery, Juntendo University, Tokyo, Japan.

The KUH Neurosurgery Department provides full-time acute and elective neurosurgical services for the KUH patient population from a recruitment area of Eastern Finland. The Kuopio NPH registry comprises all presumed iNPH patients from the KUH patient population and contains clinical baseline and follow-up data, diagnoses from other hospitals, medications and causes of death from national registries, and neuropathological findings of these patients [24,25]. The

Table 1 Comparison between the patients with Alzheimer's disease, Parkinson's disease, idiopathic normal-pressure hydrocephalus and controls in the Japanese cohort series

	NC (<i>n</i> = 10)	AD (<i>n</i> = 12)	PD (<i>n</i> = 12)	iNPH (<i>n</i> = 30)	<i>P</i>
Age, years	73 (66.5–80.5)	74.5 (72–80.5)	72 (66.25–79)	74.5 (72–80.5)	0.809
Men, <i>n</i> (%)	5 (50)	6 (50)	6 (50)	23 (77)	0.308
MMSE scores at entry	28 (28–30)	22.5 (20–27)	26 (25–29.25)	22 (20–26)	<i>P</i> 1: 0.002**, <i>P</i> 2: 1.000, <i>P</i> 3: <0.001***, <i>P</i> 4: 0.488, <i>P</i> 5: 0.702
CSF biomarker, mean (SD)					
PTPRQ, pg/ml	296 (94)	365 (225)	338 (230)	619 (398)	<i>P</i> 1: 0.921, <i>P</i> 2: 0.994, <i>P</i> 3: 0.001**, <i>P</i> 4: 0.077, <i>P</i> 5: 0.042*
Aβ42, pg/ml	633 (217)	420 (145)	657 (241)	500 (236)	<i>P</i> 1: 0.103, <i>P</i> 2: 1.000, <i>P</i> 3: 0.533, <i>P</i> 4: 0.725, <i>P</i> 5: 0.395
p-tau, pg/ml	25.1 (7.5)	103 (38.5)	22.8 (8.2)	25.6 (13.0)	<i>P</i> 1: <0.001***, <i>P</i> 2: 0.986, <i>P</i> 3: 1.000, <i>P</i> 4: <0.001***, <i>P</i> 5: 0.960
t-tau, pg/ml	170 (86)	595 (276)	116 (120)	117 (90.7)	<i>P</i> 1: 0.001**, <i>P</i> 2: 0.797, <i>P</i> 3: 0.513, <i>P</i> 4: <0.001***, <i>P</i> 5: 1.000

Aβ42, amyloid β 1-42; AD, Alzheimer's disease; CSF, cerebrospinal fluid; iNPH, idiopathic normal-pressure hydrocephalus; MMSE, Mini-Mental State Examination; NC, normal control (neurologically healthy); PD, Parkinson's disease; p-tau, tau phosphorylated at threonine 181; PTPRQ, protein tyrosine phosphatase receptor type Q; t-tau, total tau. Data are expressed as median (25%–75%), unless otherwise indicated; **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P*1, comparison between NC and AD groups; *P*2, comparison between NC and PD groups; *P*3, comparison between NC and iNPH groups; *P*4, comparison between iNPH and AD groups; *P*5, comparison between iNPH and PD groups.

Finnish cohort included the Finnish NC group and patients with probable iNPH. The Finnish NC group consisted of 30 patients (median age 71 years; 13 men) who were scheduled for knee or hip replacement surgeries at the KUH. The Finnish iNPH group consisted of 30 consecutive patients (median age 73 years; 19 men) who consented to a lumbar puncture with CSF removal. The Finnish iNPH group had undergone ventriculoperitoneal shunt with a right frontal cortical biopsy [24,26] at KUH between December 2013 and February 2015. Patients in the Finnish cohort who underwent biopsy at the time of CSF shunting were classified into two groups based on the presence or absence of amyloid and/or tau deposition in the frontal lobe cortex following histological examination (Table S1) [27–30].

Expression analysis using autopsied brains was performed during the second part of the study. Immunohistochemical staining was performed to identify the expression levels of PTPRQ in the frontal lobe cortex, cingulate gyrus at the level of mamillary bodies, hippocampus and thalamus, including the paraventricular

system of the autopsied brains of patients with iNPH (10 patients: Japanese-iNPH [*n* = 3], Finnish iNPH [*n* = 7]) and NC subjects (*n* = 6) without significant AD pathology [28–30] (Table S2). All patients were clinically followed up until death, and the development of symptoms was documented by neurologists, neurosurgeons, and general practitioners.

Patients were diagnosed with dementia according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, criteria [31]. Cognitive status was evaluated at the time of surgery and retrospectively at the time of death. Cognition was classified as normal, mild cognitive impairment, mild dementia, moderate dementia, or severe dementia (bedridden).

All clinical data from the hospitals in Juntendo University Hospital or the KUH catchment areas were collected.

Collection of cerebrospinal fluid

Cerebrospinal fluid was collected via lumbar puncture with the patient in the recumbent position (sitting

Table 2 Group comparison between idiopathic normal-pressure hydrocephalus (NPH) and non-idiopathic NPH

	iNPH (<i>n</i> = 30)	Non-idiopathic NPH (① + ②, <i>n</i> = 16)	<i>P</i> value comparison between idiopathic and non-idiopathic	① NPH with acquired aetiologies (<i>n</i> = 11)	② NPH with congenital/ developmental aetiologies (<i>n</i> = 5)	<i>P</i> value for multiple comparisons
Age, years	74.5 (72–80.5)	71.5 (66.25–75.5)	0.182	72 (67–76)	67 (62–76)	0.125
Men, <i>n</i> (%)	23 (77)	5 (31)	0.267	2 (18)	3 (60)	0.007**
Mean (SD)	35.3 (4.3)	39.2 (4.7)	0.011*	38.5 (4.6)	40.0 (5.2)	0.035*
Evans Index, %						
MMSE scores at entry	22 (20–26)	20 (15–24)	0.716	20 (14.25–20.75)	26 (21–28)	0.015*
iNPHGS total score	5 (5–7)	5 (4.5–8.5)	0.870	7 (5–9.75)	5 (3–5)	0.048*
Gait disturbance score	2 (2–2.25)	2 (1–3)	0.076	3 (1.25–3.75)	2 (1–2)	0.079
Cognitive impairment score	2 (1–3)	2 (1.5–3)	0.256	3 (2–3)	1 (0.5–2.5)	0.024*
Urinary impairment score	2 (1–2)	1 (1–2.5)	0.857	1.5 (1–3)	1 (1–1.5)	0.424
CSF biomarker, mean (SD)						
PTPRQ, pg/ml	619 (398)	1704 (861)	<0.001***	1697 (969)	1720 (656)	<i>P</i> ₁ : 0.013*, <i>P</i> ₂ : 0.053
Aβ42, pg/ ml	500 (236)	444 (204)	0.518	383 (146)	576 (266)	<i>P</i> ₁ : 0.192, <i>P</i> ₂ : 0.464
p-tau, pg/ ml	25.6 (13.0)	22.4 (11.5)	0.229	19.4 (6.6)	29 (17.6)	<i>P</i> ₁ : 0.150, <i>P</i> ₂ : 0.972
t-tau, pg/ ml	117 (90.7)	237 (184)	0.004**	216 (148)	280 (260)	<i>P</i> ₁ : 0.162, <i>P</i> ₂ : 0.551

CSF, cerebrospinal fluid; iNPH, idiopathic normal-pressure hydrocephalus; iNPHGS, idiopathic normal-pressure hydrocephalus grading scale; MMSE, Mini Mental State Examination; NPH, normal-pressure hydrocephalus; p-tau, tau phosphorylated at threonine 181; PTPRQ, protein tyrosine phosphatase receptor type Q; t-tau, total tau. Data are expressed as median (25%–75%), unless otherwise indicated; **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P*₁, comparison between iNPH and NPH with acquired aetiologies; *P*₂, comparison between iNPH and NPH with congenital/developmental aetiologies.

position for the Finnish iNPH group). All CSF samples were centrifuged to remove cells and debris, aliquoted, and immediately stored in polypropylene tubes at –80°C until biochemical analysis.

Enzyme-linked immunosorbent assay

We measured the concentration of PTPRQ in the CSF of patients with probable iNPH and in NCs using an enzyme-linked immunosorbent assay (ELISA; Cloud-Clone Corp., Houston, TX, USA) according to the manufacturer's protocol.

Additionally, we used Innostest assays for the quantitative determination of other CSF biomarkers, including p-tau (NIPRO, Osaka, Japan or Fujirebio, Ghent, Belgium), t-tau (NIPRO or Fujirebio), and Aβ42 (Fujirebio), which are markers of AD pathology. We also analysed the sensitivity and specificity of PTPRQ for the auxiliary diagnosis of probable iNPH.

Immunohistochemistry and histological evaluation

Post-mortem brain tissues were fixed in 10% neutral formalin for at least 1 week and then cut into 1-cm-thick coronal slices. The brain specimens were taken from 16 standard regions [25], embedded in paraffin, and cut into 6-μm sections. The sections were dewaxed using xylene, rehydrated in a graded series of alcohol to water, and subjected to antigen retrieval using Dako Target Retrieval Solution, Citrate pH 6 (×10; Agilent Technologies, Inc., Santa Clara, CA, USA) in an autoclave at 121°C for 10 min. Endogenous peroxidase was blocked by incubation of the brain sections with 0.3% hydrogen peroxide for 30 min. The sections were blocked with 5 × SEA BLOCK Blocking Buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 1% donkey serum phosphate-buffered saline (PBS) at 25°C for 30 min to prevent non-specific antibody binding.

The sections were incubated with rabbit polyclonal IgG anti-PTPRQ (immunized with amino acids 2208–2299; 1:50 dilution; Cloud-Clone Corp.) overnight at 4°C. The following day, the sections were incubated with EnVision System Labelled Polymer (DAKO, Glostrup, Denmark), which was used as the secondary antibody, for 30 min at room temperature. Following each treatment, the slides were washed with PBS (three times for 5 min). Finally, the sections were stained with 3,3'-diaminobenzidine (DAB) and counterstained with Mayer's haematoxylin, dehydrated, cleared and mounted.

Protein tyrosine phosphatase receptor type Q staining was viewed under an E800 microscope (Nikon, Tokyo, Japan), and images were captured with an AxioCam HRc CCD camera using AxioVision Rel 4.7.2.0 imaging processing software (Carl Zeiss, Microimaging GmbH, Jena, Germany). Whole-slide images of A β 42, tau, and haematoxylin and eosin stains were captured with a NanoZoomer Digital slide scanner (Hamamatsu Photonics K.K., Hamamatsu City, Japan) [32,33]. An experienced pathologist evaluated all the slides without knowledge of the patient's outcome.

Immunofluorescence staining

The sections were blocked using a method similar to that used for single immunohistochemical staining, and stained with rabbit polyclonal IgG anti-PTPRQ (1:50) overnight at 4°C. The following day, the sections were incubated with secondary antibodies, including goat anti-rabbit IgG (H + L) Alexa Fluor™ Plus488 (A32731; diluted 1:100, Thermo Fisher Scientific Inc.), for 60 min at room temperature. Next, the nuclei in the sections were stained with Hoechst 33342, trihydrochloride, trihydrate (H3570; Thermo Fisher Scientific Inc.) for 2 min at room temperature, and a glass coverslip was mounted onto each section using VECTASHIELD Mounting Medium (H-1000; Vector Laboratories Inc., Burlingame, CA, USA). Finally, the sections were examined under a confocal scanning microscope (Leica TCS-SP5) using a Leica Application SuiteX 3.4.2.18368 (Leica Microsystems, GmbH, Wetzlar, Germany).

In situ hybridization

In situ hybridization was performed on formalin-fixed, paraffin-embedded sections of periventricular samples in the iNPH and NC groups using the GeneticLab QuantiGene ViewRNA *in situ* hybridization tissue evaluation kit (Thermo Fisher Scientific Inc.). After deparaffinization, the sections were boiled in

pretreatment solution for 10 min, digested with protease for 20 min, and then hybridized with designed probes against PTPRQ (VA1-3017466). Fast Red substrates were used to produce signals. The sections were examined under an E800 microscope (Nikon), and images were captured with an AxioCam HRc CCD camera using AxioVision Rel 4.7.2.0 imaging processing software (Carl Zeiss, Microimaging GmbH).

Data analyses and statistics

Descriptive statistics were used to summarize the data. The Shapiro–Wilk test and QQ plots were used to assess the normality of distributions. In each comparison, we used Tamhane's T2 multiple comparison test after a one-way analysis of variance for continuous variables and Kruskal–Wallis's *H* test for categorical variables. The Mann–Whitney *U*-test with Bonferroni correction was used to compare among the different groups. Wilcoxon signed-rank test was used for within-group comparisons of MMSE and iNPHGS scores before and 3 months after CSF shunting. Pearson correlation coefficient was estimated for finding linear association between two continuous variables. A chi-squared test was conducted to compare proportions.

The receiver-operating characteristic (ROC) curve was calculated to evaluate the goodness-of-fit for predictors of probable iNPH versus healthy neurological state. Statistical analyses were performed using IBM Statistical Package for the Social Sciences Version 25.0 (SPSS, Cary, NC, USA) for Windows. For all analyses, $P < 0.05$ was considered statistically significant.

Results

Differentiation of iNPH from other neurodegenerative diseases

We confirmed that the preoperative CSF concentrations of p-tau, t-tau, and A β 42 did not differ significantly between the Japanese iNPH and the NC groups (Table 1). Conversely, CSF PTPRQ concentrations were significantly higher in the Japanese and Finnish iNPH groups (mean [SD] 619 [398] pg/ml and 762 [570] pg/ml, respectively) compared to the respective NC groups (296 [94] pg/ml, $**P = 0.001$; and 371 [94] pg/ml, $***P < 0.001$, respectively) and in the PD group (338 [230] pg/ml, $*P = 0.041$). However, the Japanese iNPH and AD groups showed no significant difference in CSF PTPRQ concentrations (619 [398] pg/ml vs. 365 [225] pg/ml; $P = 0.075$ [Table 1, Fig. 2]).

Using a cut-off PTPRQ concentration of 313 pg/ml, iNPH was detected with a sensitivity of 83.3%, specificity of 80%, and an area under the ROC curve (AUC) of 0.860 in the Japanese iNPH group. In the validation analysis, using a cut-off PTPRQ concentration of 555 pg/ml, iNPH was detected with a sensitivity of 63.3%, specificity of 100%, and an AUC of 0.849 in the Finnish iNPH group (Fig. S1).

Difference between iNPH with and without Alzheimer's disease comorbidity

Analysis of the pathological tissues obtained by cortical biopsy at the time of CSF shunting showed that there were significant differences in age ($*P = 0.01$), MMSE scores at biopsy ($*P = 0.012$), MMSE scores after shunt ($**P = 0.003$), iNPHGS scores at biopsy ($*P = 0.04$), iNPHGS scores after shunt ($*P = 0.018$), concentrations of A β 42 ($**P = 0.004$) and p-tau ($*P = 0.048$) between the group with amyloid deposits and/or tau deposits ($n = 16$) and the group without deposits ($n = 14$). However, the concentration of PTPRQ and t-tau did not differ significantly between the two groups ($P = 0.257$ and $P = 0.525$, respectively; Table S1).

Difference of PTPRQ between shunt responders and non-responders

We confirmed that there were no statistically significant differences between the Japanese and Finnish iNPH groups. The Japanese and Finnish iNPH groups were thus combined to create a single probable iNPH group, and no significant differences were found in PTPRQ concentrations between the shunt responder group ($n = 46$) and shunt non-responder group ($n = 14$; $P = 0.432$ [Table S3]).

Differences between iNPH and non-idiopathic NPH

The patients with non-idiopathic NPH (acquired + congenital/developmental) had significantly dilated ventricles compared to those with iNPH ($*P = 0.011$; Table 2). CSF PTPRQ concentrations were significantly higher in non-idiopathic NPH (mean [SD] 1704 [861] pg/ml) compared to the iNPH group ($***P < 0.001$; Fig. 3). There was a positive correlation between the Evans Index and CSF PTPRQ concentrations in NPH (correlation coefficient = 0.384, $*P = 0.013$).

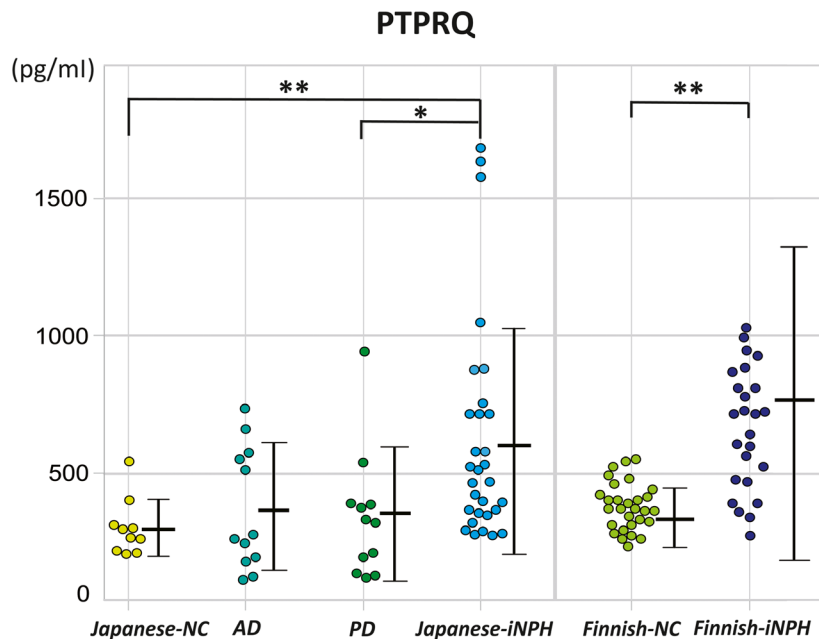


Figure 2 Group comparison of protein tyrosine phosphatase receptor type Q (PTPRQ) concentrations in the cerebrospinal fluid (CSF) determined by enzyme-linked immunosorbent assay. PTPRQ concentrations in the CSF determined by ELISA. Comparisons of CSF biomarkers between neurologically healthy individuals (normal control [NC] group), Alzheimer's disease (AD), Parkinson's disease (PD), Japanese patients with probable idiopathic normal-pressure hydrocephalus (Japanese iNPH), and Finnish patients with iNPH (Finnish iNPH) groups are shown. The middle line represents the mean, while the upper and lower edges represent the SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. [Colour figure can be viewed at wileyonlinelibrary.com]

Validation of PTPRQ expression in the brain tissues

Immunohistochemistry was performed to examine the localization of PTPRQ in the brain. We performed an absorption test to identify the specificity of PTPRQ immunostaining, and the results demonstrated that the addition of the antigen peptide to the primary antibody solution completely abolished PTPRQ immunostaining. The choroid plexus (Fig. 4a,b) and ependymal cells in patients with iNPH (Fig. 4c–e) were positively stained with the anti-PTPRQ antibody, as was the surface area of the ventricle that was directly in contact with the CSF in both iNPH and NC subjects.

Immunostaining and *in situ* hybridization also revealed that PTPRQ was expressed in the ependymal cells (Fig. 4f) and choroid plexus (Fig. 4g) of iNPH subjects.

Discussion

The phosphorylation/dephosphorylation of phosphotyrosine in proteins, and of the inositol phosphate moiety of phosphatidylinositol phospholipids, are important mechanisms through which cells regulate proliferation, survival, migration and secretion. In particular, PTPRQ is upregulated in a model of renal injury [34]. In this study, we confirmed that CSF PTPRQ levels in patients with probable iNPH were

significantly increased compared to NCs and other neurodegenerative diseases. Further, the PTPRQ concentration in non-idiopathic NPH, which generally showed more extensive ventricular enlargement than iNPH, was higher than in iNPH. In addition, the non-iNPH patients had significantly dilated ventricles compared to those with iNPH.

This was the first study to demonstrate the expression of PTPRQ in the human brains of patients with iNPH. We showed that the expression of PTPRQ in pathological tissues was confined to the ependymal cells and choroid plexus, which are directly in contact with the CSF. PTPRQ includes an extracellular domain that contains 18 fibronectin III repeats, a membrane-spanning domain, and a cytoplasmic domain with phosphatidylinositol phosphatase activity [34,35]. PTPRQ is a membrane protein localized to the apical surface and lower region of the hair bundle of inner ear hair cells, and the extracellular domain of PTPRQ has been reported to be involved in regulating the function of the hair cell bundle [36]. Therefore, we hypothesize that ependymal cells and choroidal ependymal cells are involved in actin polymerization and ciliary movement, and these ciliary structures have an important role in CSF dynamics [37,38].

The concentrations of other CSF biomarkers that have been used for the diagnosis of iNPH, such as A β 42 and tau, were lower in patients with iNPH than in healthy subjects. The reduced levels of A β 42 observed in iNPH cases may be attributable to a general reduction in the production of amyloid precursor protein-derived proteins, and this may be attributable to reduced brain metabolism in the periventricular zone as seen in positron-emission tomography and MRI studies [39]. Additionally, the observed reduction in A β peptide and tau protein levels may be explained by a reduced clearance from the extracellular fluid due to a reduced centripetal flow of extracellular fluid caused by the reversed CSF dynamics in iNPH. In addition, studies have reported that reduced CSF A β 42 in iNPH is associated with A β pathology in the brain [40,41]. In the present study, a high concentration of PTPRQ was observed in the CSF of patients with probable iNPH and non-idiopathic NPH. It is highly possible that the elevated PTPRQ levels in CSF are related to ependymal dysfunction from ventricular expansion. This finding may be useful for the diagnosis of NPH. However, the underlying cause of increased PTPRQ expression in NPH requires further research.

This study had several limitations. First, selection bias may have affected the results of this two-cohort retrospective study. In addition, the sample size was small, and this may have increased the rate of type II

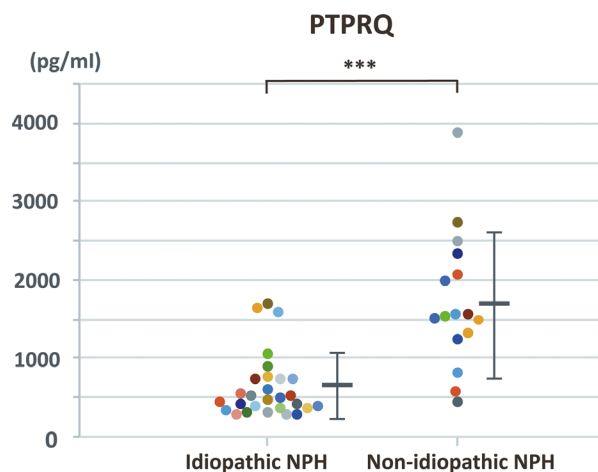


Figure 3 Group comparison of protein tyrosine phosphatase receptor type Q (PTPRQ) concentrations in the cerebrospinal fluid (CSF) between idiopathic normal-pressure hydrocephalus (iNPH) and non-idiopathic NPH. Comparisons of PTPRQ concentrations in the CSF between Japanese patients with probable iNPH and non-idiopathic NPH (acquired aetiologies + congenital/developmental aetiologies). CSF PTPRQ concentrations were significantly higher in non-idiopathic NPH (mean [SD] 1704 [861] pg/ml) compared to the iNPH group (** $P = 0.002$). [Colour figure can be viewed at wileyonlinelibrary.com]

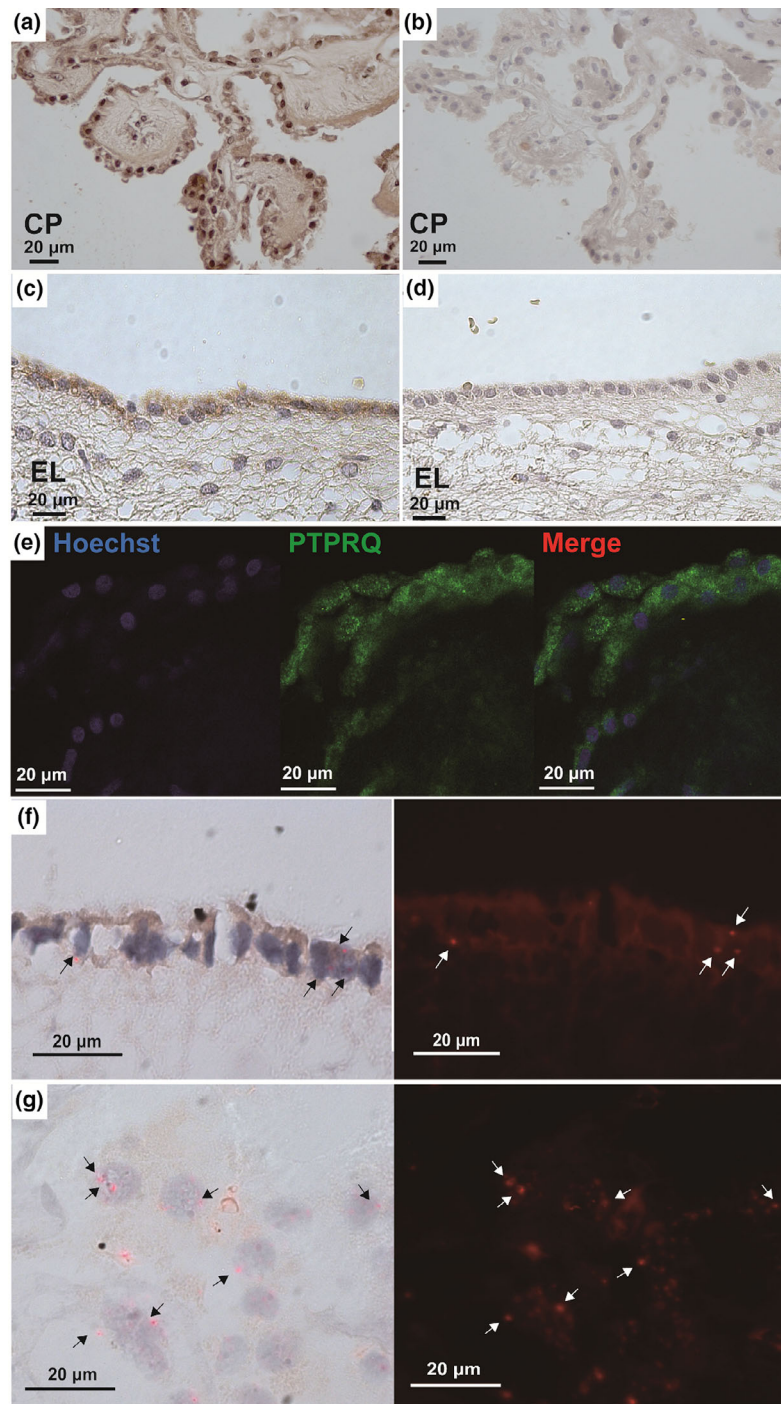


Figure 4 Protein tyrosine phosphatase receptor type Q (PTPRQ) immunohistochemistry and mRNA *in situ* hybridization. Immunohistochemistry of the post-mortem brain tissue using anti-PTPRQ antibodies. (a) and (b) are serial sections of the choroid plexus (CP), and (c), (d) and (e) are serial sections of the ependymal lining (EL) of the third ventricle in patients with idiopathic normal-pressure hydrocephalus (iNPH). PTPRQ immunostaining (a, c) is completely abolished following addition of the antigen peptide to the primary antibody solution (b, d). (a), (b), (c) and (d) are serial sections in case 10 (Tables S1–S3). (e) The ependymal cells were immunofluorescently stained with anti-PTPRQ antibody in case 8. The nuclei in the ependymal cells were immunostained with anti-PTPRQ antibody and Hoechst, and the sections were examined under a confocal scanning microscope. Scale bar = 20 μm . The results from mRNA *in situ* hybridization were analysed by counting existing dots of mRNA in patients with iNPH (Tables S1–S3: case 8, male, 72 years). PTPRQ mRNA was expressed in the cytoplasm of ependymal cells (f) and the CP (g) in iNPH patients (black and white arrows). Each red dot (black/white arrow) indicates one mRNA copy. Scale bar = 20 μm .

errors. Additionally, the evaluation period after the CSF shunt was only 3 months. In previous studies, the CSF concentrations of p-tau were higher and the CSF concentrations of A β 42 were lower in the shunt non-responder than in the shunt responder group [42,43]. Further, these differences were observed for more than 2 years. However, even if AD pathology coexisted with iNPH, we observed an improvement in symptoms at 3 months after performing the shunt [9]. Finally, the low specificity in the statistical analysis PTPRQ levels does not allow its use as a strong differential diagnostic criterion.

Nagata et al. [16] reported that PTPRQ concentrations were lower in AD patients than in iNPH patients; however, as revealed by the brain biopsy results of patients with iNPH in the present study, amyloid and tau were discovered in the frontal lobe of patients, and there were no statistically significant differences in PTPRQ concentrations between probable iNPH patients with and without AD pathology. Furthermore, there were no significant differences between the iNPH and AD groups in the first cohort. Further studies with larger samples are required to evaluate the relationship between PTPRQ concentrations and CSF shunting outcomes.

Overall, our findings demonstrated that PTPRQ levels were significantly higher in patients with probable iNPH than in NCs, regardless of the population of origin. Therefore, measurements of PTPRQ may be used for the auxiliary diagnosis of NPH.

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Disclosure of conflicts of interest

The authors declare that they have no financial or other conflicts of interest.

Data availability statement

All data not published within the article are available, and will be shared in anonymized form upon request from any qualified investigator.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Receiver-operating characteristic (ROC) curves to separate idiopathic normal-pressure hydrocephalus (iNPH) and protein tyrosine phosphatase receptor type Q. ROC curves are given for control vs. iNPH.

Table S1. Comparison of pathological findings between amyloid β and/or tau deposit-positive and -negative in the Finnish cohort.

Table S2. Baseline data for post-mortem evaluation.

Table S3. Comparison of shunt responders and shunt non-responders before and after cerebrospinal fluid shunt.

References

1. Adams RD, Fisher CM, Hakim S, Ojemann RG, Sweet WH. Symptomatic occult hydrocephalus with "normal" cerebrospinal-fluid pressure. A treatable syndrome. *N Engl J Med* 1965; **273**: 117–126.
2. Johanson CE, Duncan JA 3rd, Klinge PM, Brinker T, Stopa EG, Silverberg GD. Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. *Cerebrospinal Fluid Res* 2008; **5**: 10.
3. Silverberg GD. Normal pressure hydrocephalus (NPH): ischaemia. CSF stagnation or both. *Brain* 2004; **127**: 947–948.
4. Silverberg GD, Huhn S, Jaffe RA, et al. Downregulation of cerebrospinal fluid production in patients with chronic hydrocephalus. *J Neurosurg* 2002; **97**: 1271–1275.
5. Hashimoto M, Ishikawa M, Mori E, Kuwana N. Study of INPH on neurological improvement (SINPHONI). Diagnosis of idiopathic normal pressure hydrocephalus is supported by MRI-based scheme: a prospective cohort study. *Cerebrospinal Fluid Res* 2010; **7**: 18.
6. Mori E, Ishikawa M, Kato T, et al. Guidelines for management of idiopathic normal pressure hydrocephalus:

- second edition. *Neurol Med Chir (Tokyo)* 2012; **52**: 775–809.
7. Paterson RW, Slattery CF, Poole T, *et al.* Cerebrospinal fluid in the differential diagnosis of Alzheimer's disease: clinical utility of an extended panel of biomarkers in a specialist cognitive clinic. *Alzheimers Res Ther* 2018; **10**: 32.
 8. Shaw LM, Vanderstichele H, Knapik-Czajka M, *et al.* Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* 2009; **65**: 403–413.
 9. Nakajima M, Miyajima M, Ogino I, *et al.* Preoperative phosphorylated tau concentration in the cerebrospinal fluid can predict cognitive function three years after shunt surgery in patients with idiopathic normal pressure hydrocephalus. *J Alzheimers Dis* 2018; **66**: 319–331.
 10. Agren-Wilsson A, Lekman A, Sjöberg W, *et al.* CSF biomarkers in the evaluation of idiopathic normal pressure hydrocephalus. *Acta Neurol Scand* 2007; **116**: 333–339.
 11. Jeppsson A, Wikkelso C, Blennow K, *et al.* CSF biomarkers distinguish idiopathic normal pressure hydrocephalus from its mimics. *J Neurol Neurosurg Psychiatry* 2019; **90**: 1117–1123.
 12. Kapaki EN, Paraskevas GP, Tzerakis NG, *et al.* Cerebrospinal fluid tau, phospho-tau181 and beta-amyloid1-42 in idiopathic normal pressure hydrocephalus: a discrimination from Alzheimer's disease. *Eur J Neurol* 2007; **14**: 168–173.
 13. Pyykkö OT, Lumela M, Rummukainen J, *et al.* Cerebrospinal fluid biomarker and brain biopsy findings in idiopathic normal pressure hydrocephalus. *PLoS One* 2014; **9**: e91974.
 14. Graff-Radford NR. Alzheimer CSF biomarkers may be misleading in normal-pressure hydrocephalus. *Neurology* 2014; **83**: 1573–1575.
 15. Jeppsson A, Höltta M, Zetterberg H, Blennow K, Wikkelso C, Tullberg M. Amyloid mis-metabolism in idiopathic normal pressure hydrocephalus. *Fluids Barriers CNS* 2016; **13**: 13.
 16. Nagata Y, Bundo M, Sugiura S, *et al.* PTPRQ as a potential biomarker for idiopathic normal pressure hydrocephalus. *Mol Med Rep* 2017; **16**: 3034–3040.
 17. Shahin H, Rahil M, Abu Rayan A, *et al.* Nonsense mutation of the stereociliar membrane protein gene PTPRQ in human hearing loss DFNB84. *J Med Genet* 2010; **47**: 643–645.
 18. Nayak G, Goodyear RJ, Legan PK, Noda M, Richardson GP. Evidence for multiple, developmentally regulated isoforms of Ptpqr on hair cells of the inner ear. *Dev Neurobiol* 2011; **71**: 129–141.
 19. Kageyama H, Miyajima M, Ogino I, *et al.* Panventriculomegaly with a wide foramen of Magendie and large cisterna magna. *J Neurosurg* 2016; **124**: 1858–1866.
 20. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiat Res* 1975; **12**: 189–198.
 21. Kubo Y, Kazui H, Yoshida T, *et al.* Validation of grading scale for evaluating symptoms of idiopathic normal-pressure hydrocephalus. *Dement Geriatr Cogn Disord* 2008; **25**: 37–45.
 22. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984; **34**: 939–944.
 23. Postuma RB, Berg D, Stern M, *et al.* MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 2015; **30**: 1591–601.
 24. Leinonen V, Alafuzoff I, Aalto S, *et al.* Assessment of beta-amyloid in a frontal cortical brain biopsy specimen and by positron emission tomography with carbon 11-labeled Pittsburgh Compound B. *Arch Neurol* 2008; **65**: 1304–1309.
 25. Leinonen V, Koivisto AM, Savolainen S, *et al.* Post-mortem findings in 10 patients with presumed normal-pressure hydrocephalus and review of the literature. *Neuropathol Appl Neurobiol* 2012; **38**: 72–86.
 26. Kokkonen N, Herukka SK, Huilaja L, *et al.* Increased levels of the bullous pemphigoid BP180 autoantibody are associated with more severe dementia in Alzheimer's disease. *J Invest Dermatol* 2017; **137**: 71–76.
 27. Leinonen V, Rauramaa T, Johansson J, *et al.* S-[18F]THK-5117-PET and [11C]PIB-PET imaging in idiopathic normal pressure hydrocephalus in relation to confirmed amyloid-beta plaques and tau in brain biopsies. *J Alzheimers Dis* 2018; **64**: 171–179.
 28. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991; **82**: 239–259.
 29. Thal DR, Rüb U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002; **58**: 1791–1800.
 30. Thal DR, Capetillo-Zarate E, Del Tredici K, Braak H. The development of amyloid beta protein deposits in the aged brain. *Sci Aging Knowledge Environ* 2006; **2006**: re1.
 31. Frances A, First MB, Pincus HA. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. American Psychiatric Association: Washington DC, 1994.
 32. Halama N, Zoernig I, Spille A, *et al.* Quantification of prognostic immune cell markers in colorectal cancer using whole slide imaging tumor maps. *Anal Quant Cytol Histol* 2010; **32**: 333–340.
 33. Nielsen PS, Riber-Hansen R, Jensen TO, Schmidt H, Steiniche T. Proliferation indices of phosphohistone H3 and Ki67: strong prognostic markers in a consecutive cohort with stage I/II melanoma. *Mod Pathol* 2013; **26**: 404–413.
 34. Oganessian A, Poot M, Daum G, *et al.* Protein tyrosine phosphatase RQ is a phosphatidylinositol phosphatase that can regulate cell survival and proliferation. *Proc Natl Acad Sci USA* 2003; **100**: 7563–7568.
 35. Wright MB, Hugo C, Seifert R, Distèche CM, Bowen-Pope DF. Proliferating and migrating mesangial cells responding to injury express a novel receptor protein-tyrosine phosphatase in experimental mesangial proliferative glomerulonephritis. *J Biol Chem* 1998; **273**: 23929–23937.
 36. Hirono M, Denis CS, Richardson GP, Gillespie PG. Hair cells require phosphatidylinositol 4,5-bisphosphate for mechanical transduction and adaptation. *Neuron* 2004; **44**: 309–320.
 37. Banizs B, Pike MM, Millican CL, *et al.* Dysfunctional cilia lead to altered ependyma and choroid plexus

- function, and result in the formation of hydrocephalus. *Development* 2005; **132**: 5329–5339.
38. Narita K, Takeda S. Cilia in the choroid plexus: their roles in hydrocephalus and beyond. *Front Cell Neurosci* 2015; **9**: 39.
 39. Momjian S, Oowler BK, Czosnyka Z, Czosnyka M, Pena A, Pickard JD. Pattern of white matter regional cerebral blood flow and autoregulation in normal pressure hydrocephalus. *Brain* 2004; **127**: 965–972.
 40. Elobeid A, Laurell K, Cesarini KG, Alafuzoff I. Correlations between mini-mental state examination score, cerebrospinal fluid biomarkers, and pathology observed in brain biopsies of patients with normal-pressure hydrocephalus. *J Neuropathol Exp Neurol* 2015; **74**: 470–479.
 41. Seppälä TT, Nerg O, Koivisto AM, *et al.* CSF biomarkers for Alzheimer disease correlate with cortical brain biopsy findings. *Neurology* 2012; **78**: 1568–1575.
 42. Moriya M, Miyajima M, Nakajima M, Ogino I, Arai H. Impact of cerebrospinal fluid shunting for idiopathic normal pressure hydrocephalus on the amyloid cascade. *PLoS One* 2015; **10**: e0119973.
 43. Nakajima M, Miyajima M, Ogino I, *et al.* Cerebrospinal fluid biomarkers for prognosis of long-term cognitive treatment outcomes in patients with idiopathic normal pressure hydrocephalus. *J Neurol Sci* 2015; **357**: 88–95.