Idiopathic normal pressure hydrocephalus is a slowly progressive syndrome in the elderly characterized by gait disorder, cognitive deterioration, and urinary incontinence. Compared to earlier studies, a higher annual incidence of the syndrome was noted in a Finnish population with an increasing trend. High vascular comorbidity and mortality was observed without a major neuroinflammatory component. Apolipoprotein E genotypes did not differentiate patients with idiopathic normal pressure hydrocephalus from healthy age- and gender-matched controls.
Idiopathic normal pressure hydrocephalus: a study of epidemiology, genetics, and cerebrospinal fluid
OKKO T. PYYKKÖ

Idiopathic normal pressure hydrocephalus: a study of epidemiology, genetics, and cerebrospinal fluid

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ABSTRACT

Idiopathic normal pressure hydrocephalus (iNPH) is a slowly progressive syndrome in the elderly characterized by gait disorder, cognitive deterioration, and urinary incontinence. Symptoms of iNPH can be relieved and even reversed by ventriculoperitoneal shunt surgery in most patients. The aim of this thesis was to investigate incidence, comorbidities, mortality, and causes of death in iNPH. In addition, the potential effects of apolipoprotein E (APOE) genotypes in the diagnostics and prognostics in iNPH was explored. Another aim was to evaluate a large panel of cerebrospinal fluid (CSF) biomarkers in iNPH and how they relate to brain biopsy findings.

Kuopio NPH registry consists of all evaluated possible iNPH patients from the Kuopio University Hospital catchment population since 1993 and contains clinical baseline and follow-up data, other hospital diagnoses, and medications. In addition, all patients in the registry underwent a frontal cortical biopsy during a diagnostic intracranial pressure monitoring or shunt surgery. Annual population of the catchment area and causes of death were obtained from the national registries. Patients with a blood sample available for DNA extraction were genotyped for APOE. Furthermore, lumbar and ventricular CSF samples were analyzed for several biomarkers: beta amyloid (Aβ) isoforms, soluble amyloid precursor protein (sAPP) isoforms, proinflammatory cytokines, and biomarkers of neuronal damage.

Compared to earlier studies, a higher annual incidence of iNPH was noted in Middle and Eastern Finnish populations with an increasing trend. While being relatively uncommon in the total population (1.8 / 100,000 / year), a much higher age-specific cumulative incidence of iNPH was seen in persons aged 70 or older (15 / 100,000 / year). The most common comorbidity in patients with iNPH was arterial hypertension. Additionally, type 2 diabetes mellitus was twice more common in patients with iNPH compared to controls (23% vs. 13%, \( p = 0.002 \)). The most common causes of death in iNPH were ischaemic heart disease (22%) and cerebrovascular disease (19%). In contrast to previous small-scale studies, APOE genotypes did not differ between 113 iNPH patients and 687 healthy elderly controls. However, APOE ε4 allele was associated with Alzheimer’s disease (OR 4.3, 95% CI 2.0–9.3) and presence of Aβ in the brain biopsy (OR 8.7, 95% CI 3.8–20), which is in line with previous studies. Decreased levels of sAPP alpha in the CSF were identified as a potential diagnostic biomarker for iNPH. No neuroinflammatory findings were identified in the CSF samples of patients with iNPH.
Idiapaitainen normaalipaineinen hydrokefalus (iNPH) on iäkkäillä esiintyvä hitaasti etenevä oireyhtymä, jonka tyyppioireita ovat kävelyvaikeudet, kognitiivinen heikentyminen ja virtsan-pidätysvaikeus. Useimmilla potilailla oireistoa voidaan helpottaa tai jopa parantaa kokonaan leik-kauksella, jossa sunttikatetri viedään aivokammiosta vatsaonteloon. Tämän väitöskirjan tavoitteena oli tutkia iNPH:n esiintyvyyttä, liitännäissairauksia, kuolleisuutta ja kuolemansyitä. Lisäksi tavoitteena oli arvioida apolipoproteiini E (APOE) -genotyyppauksen mahdollista hyötyä iNPH:n diagnostiikassa ja ennusteessa. Päämääränä oli myös arvioida laajasti aivo-selkäyrdinnestemerkiaineita iNPH-potilailla ja kuinka merkikäenteet liittyvät aivobipsian löyöksiin.


Aiempiin tutkimuksiin verraten iNPH:n ilmaantuvuus oli korkeampi keski- ja itäsuomalaisessa väestössä, mutta sairaaladiagnostiset lääketieteelliset arvokriteerit ovat määritetty useammalle väestölle. Väestö vahvisti, että iNPH:n ilmenneminen on seurataan erityisesti korkean verenpaineenäytteen varten. Aiempi tutkimus kuitenkin korostaa, että iNPH:n ilmenneminen on korkean verenpaineenäytteen varten seurataan erityisesti korkean verenpaineenäytteen varten. Aiempi tutkimus kuitenkin korostaa, että iNPH:n ilmenneminen on korkean verenpaineenäytteen varten seurataan erityisesti korkean verenpaineenäytteen varten.
One might say science is the sum total of our knowledge of the universe, the great library of the known, but the practice of science happens at the border between the known and the unknown. Standing on the shoulders of giants, we peer into the darkness with eyes opened not in fear but in wonder.

Professor Brian Cox – in Wonders of the Universe
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14th of February 2016, in Lahti

Okko T. Pyykkö

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<th>Definition</th>
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<tr>
<td>ACE/ACE</td>
<td>Angiotensin I converting enzyme gene/protein</td>
</tr>
<tr>
<td>ACZ</td>
<td>Acetazolamide</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid beta</td>
</tr>
<tr>
<td>Aβ38/40/42</td>
<td>Amyloid beta isoprotein, length 38/40/42 amino acids</td>
</tr>
<tr>
<td>APOE/apoE</td>
<td>Apolipoprotein E gene/protein</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood–brain barrier</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DESH</td>
<td>Disproportionately enlarged subarachnoid space hydrocephalus</td>
</tr>
<tr>
<td>DLB</td>
<td>Dementia with Lewy bodies</td>
</tr>
<tr>
<td>ELD</td>
<td>External lumbar drainage</td>
</tr>
<tr>
<td>ETINPH</td>
<td>Essential tremor–idiopathic normal pressure hydrocephalus syndrome</td>
</tr>
<tr>
<td>ETV</td>
<td>Endoscopic third ventriculostomy</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Haemoglobin A1c</td>
</tr>
<tr>
<td>HPt</td>
<td>Hyperphosphorylated tau protein</td>
</tr>
<tr>
<td>HSPG</td>
<td>Heparan sulfate proteoglycan</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNPH</td>
<td>Idiopathic normal pressure hydrocephalus</td>
</tr>
<tr>
<td>KUH</td>
<td>Kuopio University Hospital</td>
</tr>
<tr>
<td>LPS</td>
<td>Lumboperitoneal shunt</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein-1</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-Mental State Examination</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NFL</td>
<td>Neurofilament light protein</td>
</tr>
<tr>
<td>NPH</td>
<td>Normal pressure hydrocephalus</td>
</tr>
<tr>
<td>p-tau 181</td>
<td>Tau phosphorylated at threonine 181</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>PDD</td>
<td>Parkinson’s disease dementia</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>sAPPα/β</td>
<td>Soluble amyloid precursor protein alpha/beta</td>
</tr>
<tr>
<td>SFMBT1</td>
<td>Scm-like with four MBT domains 1 gene</td>
</tr>
<tr>
<td>sNPH</td>
<td>Secondary normal pressure hydrocephalus</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-photon emission computed tomography</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>Tf-1/Tf-2</td>
<td>Transferrin-1/transferrin-2 isoform ratio</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-alpha</td>
</tr>
<tr>
<td>VAS</td>
<td>Ventriculoatrial shunt</td>
</tr>
<tr>
<td>VCI</td>
<td>Vascular cognitive impairment</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
</tr>
<tr>
<td>VPS</td>
<td>Ventriculoperitoneal shunt</td>
</tr>
</tbody>
</table>
1 Introduction

In 1964 doctor Salomon Hakim described a syndrome of symptomatic hydrocephalus with normal cerebrospinal fluid (CSF) pressure in his thesis Some Observations on C.S.F. Pressure. Hydrocephalic Syndrome in Adults with “Normal” C.S.F. Pressure (1). The following year Hakim published his findings with Raymond D. Adams in the scientific papers (2, 3). Despite the absence of elevated pressure observed in the CSF system of the presented patients, a diversion of CSF with an implantable shunt device corrected this condition (2). While Hakim’s thesis was the first one to identify normal pressure hydrocephalus (NPH) as an independent treatable syndrome, case reports of patients with similar clinical features and findings had been reported by Foltz and Ward in 1956 (4) and McHugh in 1964 (5).

NPH is characterized by a triad of symptoms: gait disorder, cognitive impairment, and urinary incontinence in adult patients with findings of communicating hydrocephalus in a neuroradiological study of the brain and CSF pressure within normal range (2). Secondary NPH occurs after head trauma, subarachnoid haemorrhage, or other insult to the brain (6). In contrast, when no such predisposing factors are identified, the syndrome is described as primary or idiopathic NPH (iNPH) (7). The differential diagnosis of iNPH includes Alzheimer’s disease (AD), Parkinson’s disease, and cerebrovascular disease (7). Arterial hypertension is the most common comorbidity in persons with iNPH and the burden of cardiovascular risk factors is higher compared to the general population (8, 9). Unlike the differential diagnostic disorders, iNPH is an uncommon syndrome with a reported incidence between 0.5–5.5 cases per 100 000 inhabitants a year (10–15).

Although iNPH is considered to be sporadic in nature, a number of case reports of familial iNPH has emerged (16–21), supporting the potential genetic predisposition. Some previous studies have suggested a possibility of similar genetical background in iNPH and AD. An overrepresentation of the ε4 allele of the apolipoprotein E (APOE) gene was noted in one small study (22) and a better response to shunt treatment in patients with the ε3/ε3 genotype compared to patients with other genotypes was reported in another paper (23).

Diagnostic tests of CSF circulation dynamics are currently in the clinical practice of iNPH (7). In contrast, several potential CSF biomarkers have been proposed (24), but are not included in the current diagnostic criteria. In AD, determining CSF levels of amyloid beta 42 (Aβ42), hyperphosphorylated tau (HPτ) 181, and total tau are used as supplemental diagnostic tests (25), and can be utilized to differentiate AD from iNPH (26).

During the six decades after Hakim’s and Adams’ seminal publication, a significant number of papers studying iNPH have been published. While our understanding of the special clinical problem of iNPH has expanded, many questions remain unanswered. This doctoral thesis focuses on a cohort of patients from a defined geographical area referred to the Kuopio University Hospital (KUH) as suspected iNPH patients from Middle and Eastern Finland from 1993 to 2010. In addition, association of APOE genotype and various CSF biomarkers with AD and cortical brain biopsy findings are explored.
2 Review of the literature

2.1 DEFINITION OF IDIOPATHIC NORMAL PRESSURE HYDROCEPHALUS (INPH)

Idiopathic normal pressure hydrocephalus (iNPH) is typically defined as a slowly progressive clinical syndrome of gait disturbance, cognitive deterioration, and urinary incontinence in the elderly with a normotensive impairment of cerebrospinal fluid (CSF) circulation in conjunction with ventricular dilation in the absence of identified predisposing factors (27). While this is the standard definition of iNPH given in most papers, interestingly, there is currently no consensus statement on the definition in scientific literature.

2.2 CLINICAL FEATURES OF INPH

Principally, an impairment of gait, cognition, and urinary continence define the classic triad of symptoms of NPH (2) including the idiopathic type (28). Gait disturbance is the most frequent symptom in iNPH with a reported frequency between 91–100% in larger series (6, 29–32). Papers focusing on the gait analysis of iNPH describe the following frequent abnormalities: slowness, disequilibrium, decreased and variable stride length, decreased foot-to-floor clearance, broad-based, arrestments (freezing), and difficulties in turning around (dyspraxia) (33–35). Atypically, paratonic rigidity with brisk deep tendon reflexes and Babinski sign have sometimes been observed (33).

Cognitive deterioration is present in 78–88% of the iNPH patients at the time of the diagnosis (6, 29–31). Studies of neuropsychological profile of iNPH have shown impairment in several areas, notably in psychomotor speed, executive functions, attention and concentration, memory and learning, visuospatial functions, and dexterity (36–41). Clinically, the neuropsychological profile of iNPH bears a resemblance to dementia of the subcortical type (37).

Urinary incontinence is the least common symptom of the triad with a frequency of 60–90% in iNPH patients (6, 29–31). In iNPH patients, lower urinary tract symptoms include storage symptoms with urinary urgency, nocturia, and urgency incontinence being the most frequent ones; and voiding symptoms with retardation in initiating urination and prolongation/poor flow reported as the most common of the voiding symptoms (42). Urodynamic abnormalities seen in iNPH are indicative of detrusor overactivity of the bladder, and primarily explained by an exaggerated micturition reflex and frontal lobe hypoperfusion as the proposed mechanisms (42).

Importantly, even though considered to be one of the defining features of iNPH, a complete triad of symptoms is observed only in half of the iNPH patients (6, 30, 31). Additional potential symptoms of iNPH, such as hypokinetic motor deficit of the upper extremities (43), headache (44), and psychiatric symptoms of apathy, anxiety, and depression (45, 46), have also been presented in the literature.

2.3 EPIDEMIOLOGY OF INPH

2.3.1 Incidence

Generally regarded as an uncommon disease, iNPH has been in focus only in few epidemiological studies. Hospital-based studies of iNPH in the Netherlands (10), Sweden (12), Norway (14), and the United States of America (15) have reported an incidence of 0.5–1.2 shunted iNPH patients per 100,000 inhabitants per year, while in Germany an estimated annual incidence of 1.2 iNPH patients considered for surgical intervention per 100,000 inhabitants was reported in a survey-based study (11) (Table 1). An American study reported an age-specific incidence of 5.8 cases of noncongenital hydrocephalus without predisposing intracranial bleed in computed tomography (CT) scan per 100,000 inhabitants aged 65 or older (47), while a Japanese study presented an annual incidence
of 1.2 possible iNPH patients per 1000 inhabitants aged 70 or older (48) (Table 1). The diagnosis of iNPH is relatively rare in the total population, whereas it is markedly more common in the elderly. The only true population-based epidemiological study of iNPH was conducted in Norway (13). Derived from prevalence data, a minimum incidence of 5.5 probable iNPH patients per 100,000 inhabitants per year was estimated, which indicates iNPH to be an underdiagnosed or undertreated disease as most hospital-based studies reported considerably lower incidence figures (13) (Table 1). In contrast, a single paper reported no diagnosed iNPH patients in a population-based dementia study of five years in the United States of America (49) (Table 1).

2.3.2 Prevalence
Logically, most papers exploring the prevalence of iNPH study a defined population of elderly in a single community. Prevalence between 0.01–2.9% of the elderly population has been established in studies carried out in San Marino (50), Germany (51), Singapore (52), Spain (53), Japan (48, 54–56), Turkey (57), and Sweden (58) (Table 1). In total population, probable iNPH has a prevalence of 21.9 per 100,000 inhabitants as reported in a Norwegian study (13) (Table 1).

2.4 PATHOPHYSIOLOGY OF INPH

2.4.1 Cerebrospinal fluid circulation and hydrocephalus
CSF is formed in the choroid plexus, the ependyma, and the parenchyma of the ventricular system of the brain (59–61). Eighty percent of the CSF formation occurs in the choroid plexus, which comprises 60% of the total internal surface area of the ventricles (60). CSF is formed at the rate of 20 ml/h or 500 ml/day (60) by means of passive filtration across the capillary endothelium and regulated secretion across the choroidal epithelium (61).

The current classical concept of the CSF circulation, or the third circulation, was introduced by Harvey Cushing in 1926 (61, 62). CSF formed in the lateral ventricles flows through the paired interventricular foramina to the third ventricle and through the cerebral aqueduct to the fourth ventricle (59). The fluid exits the ventricular system through the median aperture and lateral apertures of the fourth ventricle into the subarachnoid space or drains through the obex to the spinal canal (59). From the subarachnoid space, CSF is absorbed into the blood circulation via the villi of the arachnoid granulations projecting into the venous sinuses (59–61). In recent years, this traditional view of CSF circulation has been challenged and more complex models of CSF turnover have been presented (61, 63).

If the rate of CSF formation and absorption is in equilibrium, the total volume of CSF in adults is 90–330 ml (60, 61, 64) with 50–100 ml in the spinal CSF space (64, 65). In the pathological states of the CSF turnover, the formation and absorption of CSF is in a state of disequilibrium leading to a disproportionally large volume of CSF intracranially, or hydrocephalus. Potential causative factors include an obstruction of CSF flow within the ventricular system or at the subarachnoid space (decreased absorption), or rarely a papilloma of the choroid plexus (increased formation) (59).

2.4.2 Pathophysiological theories
The cause of iNPH is unknown and the pathogenesis is poorly understood. Originally, Hakim and Adams hypothesized that an increase of intracranial pressure (ICP) precedes the normal pressure state dilating the ventricles and as the cause of the hydrocephalus is relieved, a new balance of CSF formation and absorption develops lowering the pressure but leaving the ventricles dilated (2). Despite the normal pressure conditions, more force is applied to the brain as the ventricular surface area is larger compared to the initial hydrocephalic phase (force = pressure × area), ultimately causing the neurologic symptoms by means of tangential shearing forces affecting the paracentral fibers of the corona radiata (2, 66).

A myriad number of pathophysiological theories for iNPH have been subsequently proposed. Currently most theories of the pathogenesis in iNPH stress the pathological CSF dynamics, the role of vascular changes, or metabolic changes related to CSF stagnation.

As the name suggests, the mean CSF pressure in iNPH is within normal range of 5–15 mmHg (2). Nevertheless, several disturbances of CSF dynamics have been reported. Intermittent rises in the CSF pressure (B-waves) (67) can be observed in a prolonged CSF pressure monitoring in iNPH
especially during sleep. In addition, abnormal ICP pulsatility has been associated with positive response to shunt treatment. Magnetic resonance imaging (MRI) studies have demonstrated CSF flow hyperdynamics, such as increased aqueductal stroke volume.

A reduced global cerebral blood flow (CBF) in iNPH was reported originally in 1969 and has been verified since in numerous studies. Additionally, a reduction in regional CBF and the presence of deep white matter ischaemia is well-documented in iNPH. Whether the abnormal cerebral circulation and vascular changes are a cause or effect of the ventricular dilation and iNPH is under debate. Some authors have suggested that dilated ventricles stretch the anterior cerebral arteries over the corpus callosum and the pathologic periventricular CSF absorption and the increase of intraparenchymal pressure leads to the reduced blood flow causing ischaemia. In contrast, it has been postulated that periventricular ischaemic injury causes decreased tensile strength in brain tissue and subsequently leads to ventriculomegaly. A more recent theory depicts arterial circulatory changes as an epiphenomena to the lowered intracranial compliance due to venous hypertension, which leads to decreased absorption of CSF via the arachnoid granulations in the superior sagittal sinus. Another theory suggests that iNPH is preceded by benign external hydrocephalus in childhood and followed by a loss of compensatory mechanisms after deep white matter ischaemia in adulthood, consequently leading to symptomatic hydrocephalus. Frequent comorbid vascular diseases reported in iNPH patients can be seen as supportive features for the vascular theories of iNPH. A single theory recognizes the presence of disturbed CSF dynamics and ischaemic changes, but hypothesizes that the stagnation and reduced turnover of CSF leads to the accumulation of amyloid beta (Aβ) and tau (τ) proteins and potentially other toxic metabolic products in the interstitial fluid of the brain parenchyma, subsequently causing dementia. In contrast to this theory, most patients with iNPH do not show Aβ or tau proteins in brain biopsy. In another theory, metabolic disturbance was proposed to be decoupled from CSF dynamics at a certain 'point of no return' of the pathogenesis of iNPH.
Table 1. Incidence and prevalence of idiopathic normal pressure hydrocephalus (iNPH) in literature.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Region</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casmiro et al. 1989</td>
<td>San Marino</td>
<td>Not specified</td>
<td>–</td>
</tr>
<tr>
<td>Vanneste et al. 1992</td>
<td>The Netherlands</td>
<td>City of Amsterdam</td>
<td>0.5 / 100,000 / year</td>
</tr>
<tr>
<td>Alexander et al. 1995</td>
<td>United States of America</td>
<td>Seattle, Washington</td>
<td>5.8* / 100,000 / year</td>
</tr>
<tr>
<td>Trenkwalder et al. 1995</td>
<td>Germany</td>
<td>Two Bavarian villages</td>
<td>–</td>
</tr>
<tr>
<td>Krauss and Halve 2004</td>
<td>Germany</td>
<td>Several</td>
<td>1.2 / 100,000 / year</td>
</tr>
<tr>
<td>Tan et al. 2004</td>
<td>Singapore</td>
<td>Several</td>
<td>–</td>
</tr>
<tr>
<td>Tisell et al. 2005</td>
<td>Sweden</td>
<td>Nationwide</td>
<td>0.9 / 100,000 / year</td>
</tr>
<tr>
<td>Knopman et al. 2006</td>
<td>United States of America</td>
<td>Rochester, Minnesota</td>
<td>0</td>
</tr>
<tr>
<td>Gascón-Bayarri et al. 2007</td>
<td>Spain</td>
<td>El Prat de Llobregat, Catalonia</td>
<td>–</td>
</tr>
<tr>
<td>Brean and Eide 2008</td>
<td>Norway</td>
<td>Vestfold County</td>
<td>5.5 / 100,000 / year</td>
</tr>
<tr>
<td>Hiraoka et al. 2008</td>
<td>Japan</td>
<td>Town of Tajiri</td>
<td>–</td>
</tr>
<tr>
<td>Arslantaş et al. 2009</td>
<td>Turkey</td>
<td>Middle Anatolia</td>
<td>–</td>
</tr>
<tr>
<td>Brean et al. 2009</td>
<td>Norway</td>
<td>Nationwide</td>
<td>1.1 / 100,000 / year</td>
</tr>
<tr>
<td>Iseki et al. 2009</td>
<td>Japan</td>
<td>Town of Takahata &amp; City of Sagae</td>
<td>–</td>
</tr>
<tr>
<td>Tanaka et al. 2009</td>
<td>Japan</td>
<td>Town of Tajiri</td>
<td>–</td>
</tr>
<tr>
<td>Klassen and Ahlskog 2011</td>
<td>United States of America</td>
<td>Olmsted County, Minnesota</td>
<td>1.2 / 100,000 / year</td>
</tr>
<tr>
<td>Iseki et al. 2014</td>
<td>Japan</td>
<td>Town of Takahata</td>
<td>1.2** / 1000 / year</td>
</tr>
<tr>
<td>Jaraj et al. 2014</td>
<td>Sweden</td>
<td>City of Gothenburg</td>
<td>–</td>
</tr>
</tbody>
</table>

*Age-specific incidence (≥65 yo inhabitants)
**Age-specific incidence (≥70 yo inhabitants)
CT = computed tomography
<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/396 (0.5%) of 67–87 yo inhabitants</td>
<td>67, 72, 77, 82, and 87 yo inhabitants evaluated</td>
</tr>
<tr>
<td>–</td>
<td>Shunted iNPH patients (n = 127)</td>
</tr>
<tr>
<td>–</td>
<td>Noncongenital hydrocephalus without predisposing intracranial bleed in CT scan (n = 8)</td>
</tr>
<tr>
<td>4/982 (0.4%) of &gt;65 yo inhabitants</td>
<td>Only inhabitants with basal ganglia symptoms (45/982) evaluated as a part of parkinsonism study</td>
</tr>
<tr>
<td>–</td>
<td>Estimation. iNPH patients considered for surgical intervention</td>
</tr>
<tr>
<td>1/14,906 (0.01%) of ≥50 yo inhabitants</td>
<td>Only inhabitants with positive Parkinson disease screening questionnaire (1246/14906) evaluated</td>
</tr>
<tr>
<td>–</td>
<td>Shunted iNPH patients (n = 243)</td>
</tr>
<tr>
<td>–</td>
<td>No NPH cases were reported during 1990–1994 in the study population</td>
</tr>
<tr>
<td>1/1754 (0.06%) of ≥70 yo inhabitants</td>
<td>Only inhabitants with dementia (165/1754) evaluated</td>
</tr>
<tr>
<td>48/219,478 (21.9 / 100,000)</td>
<td>Probable iNPH. Incidence calculated from the prevalence data.</td>
</tr>
<tr>
<td>5/170 (2.9%) of ≥65 yo inhabitants</td>
<td>Possible iNPH</td>
</tr>
<tr>
<td>1/3200 (0.03%) of ≥55 yo inhabitants</td>
<td>Only inhabitants with dementia (262/3200) evaluated</td>
</tr>
<tr>
<td>–</td>
<td>Shunted iNPH patients (n = 252)</td>
</tr>
<tr>
<td>4/790 (0.5%) of &gt;61 yo inhabitants</td>
<td>Possible iNPH</td>
</tr>
<tr>
<td>7/497 (1.4%) of ≥65 yo inhabitants</td>
<td>Possible iNPH</td>
</tr>
<tr>
<td>–</td>
<td>Shunted for iNPH (n = 13) (3.7 / 100 000 / year clinically suspected iNPH)</td>
</tr>
<tr>
<td>3/211 (1.4%) of 80 yo inhabitants</td>
<td>Possible iNPH</td>
</tr>
<tr>
<td>26/1,238 (2.1%) of ≥70 yo inhabitants</td>
<td>Probable iNPH</td>
</tr>
</tbody>
</table>
2.5 GENETIC BACKGROUND OF INPH

2.5.1 Familial occurrence
Papers exploring the genetics of iNPH are scarce, even though genetic predisposition seems conceivable, as there are several reported cases of possible familial iNPH. Three case studies have reported siblings developing a shunt-responsive iNPH with no identified contributing environmental factors (16–18). In addition, an overrepresentation of iNPH symptoms (7.1%) in the relatives of iNPH patients compared to control relatives (0.7%) have been noted and an iNPH pedigree with four affected individuals was published by the same group (20). The most recent report of potential familial iNPH presents a total of four cases in two generations with atypical clinical features (21). The largest iNPH pedigree in literature was reported in Japan with four cases of diagnosed iNPH and four individuals with suspected NPH in three generations (Figure 1) (19). Author hypothesized that in the familial subgroup of iNPH, the syndrome is inherited in an autosomal-dominant fashion (19).

A novel heritable syndrome of essential tremor–idiopathic normal pressure hydrocephalus (ETINPH) was introduced in 2008 (90). In five generations, the affected members of the kindred developed essential tremor at the ages of 16–44 years and later iNPH at the onset age of >65 years in an autosomal-dominant pattern (Figure 2) (90). In the genetical analyses, neither copy number changes nor susceptible genetic defects linked to established loci associated with tremor and Parkinson’s disease (PD) were identified (90). In the following genome-wide linkage scan, the locus of ETINPH gene was mapped to chromosome 19q12–13.31, which comprises several potential neuronal genes (91).
Figure 2. Pedigree of familial essential tremor–idiopathic normal pressure hydrocephalus (ETINPH) in three generations. Squares and circles indicate males and females, respectively. Blue symbols indicate individuals with a suspicion or diagnosis of iNPH and/or ET. The diagonal line indicates a deceased family member. Adapted from Zhang et al. 2008 (90).
2.5.2 Apolipoprotein E ε4 as a potential risk factor

Apolipoprotein E (apoE) is a 299 amino acid glycoprotein with a relative molecular weight of 34 kD (92). The main function of apoE in lipid metabolism is redistribution of lipids among cells of different organs and within an organ or tissue (92, 93). At molecular level, the function of apoE can be derived from its structure: the globular N-terminus domain (residues 1–191) contains the binding site for the low-density lipoprotein (LDL) receptor and the helical C-terminus (residues 216–299) contains the major lipid binding site (94). At cellular level, in addition to the general cholesterol redistribution, apoE participates in several neurobiological processes (93, 95).

The gene coding for apoE is located in the long arm of chromosome 19 (Figure 3) (96–98). The APOE gene consists of 3.6 kilobases with four exons and three introns (Figure 3) (95). Primarily, APOE is expressed in the liver and central nervous system (CNS) (cerebral cortex, hippocampus, cerebellum, medulla) in addition to the adrenal gland, testis, skin, kidney, spleen, adipose tissue, and various tissue macrophages (93, 95).

The polymorphism of the APOE was first discovered in 1977 utilizing isoelectric focusing (99) and expanded further with two-dimensional electrophoresis (100). Altogether three different apoE isoproteins are coded by three different APOE alleles: ε2, ε3, and ε4 (101). In the diploid karyotype, six different genotypes are possible: three homozygous (ε2/ε2, ε3/ε3, and ε4/ε4) and three heterozygous (ε2/ε3, ε2/ε4, and ε3/ε4) leading to six corresponding phenotypes (E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, and E3/E4) (101). ApoE3 is accepted as the common form and apoE2 and apoE4 as mutations differing from apoE3 at positions 112 or 158 by a single amino acid (Figure 3) (93, 102). Both sites of variation reside at the N-terminus domain of apoE (Figure 3).

The structural dissimilarity of apoE isoproteins reflects the variations in the interactions between the apoE and LDL receptor, very low-density lipoprotein (VLDL) receptor, the apoE receptor-2, and megalin (93). Isoform-specific differences in the binding affinity to heparan sulfate proteoglycans (HSPG) have been noted (93). These differences in molecular interactions translate to cellular neurobiology and pathology. Fundamentally, apoE2 and apoE3 are more neuroprotective than apoE4 (93, 103). Described functional impairments of apoE4 include reduced Aβ clearance, increased accumulation of neurofibrillary tangles, phosphorylation of tau, inhibition of neuronal outgrowth, impairment of neuronal plasticity, impairment of blood–brain barrier (BBB), reduced protection against oxidative stress, and increased neurotoxicity and neuroinflammation (93, 95, 103–105) (Figure 4).

Due to the inferior functionality of apoE4, several neurological diseases have been associated with the presence of ε4 allele. The connection between APOE genotypes and AD was described in 1993 (106). APOE ε4 allele is the major genetic risk factor for AD – it is overrepresented and advances the onset of the disease (102, 107). In other neurological disorders such as head trauma, stroke and PD, an association of ε4 allele and unfavourable outcome have been reported (105). In addition to neurological diseases, APOE ε4 has been associated with cardiovascular diseases (108).

In iNPH, an overrepresentation of ε4 allele in NPH patients (23.0%) compared to controls (6.9%) was reported in a small Italian study (22). No differences were reported in the distribution of the alpha 1-antichymotrypsin or presenillin 1 gene types in the same study (22). In the earlier cited Canadian case study (2.5.1 Familial occurrence), both affected individuals were homozygous for the ε3 allele (18). In an Icelandic preliminary report, an association with APOE ε3/ε3 genotype and a favourable gait response to shunt surgery in iNPH patients (n = 15) was noted (23), however, no follow-up study was published.
Figure 3. Locus of the apolipoprotein E (APOE) gene in chromosome 19 and two sites of variation responsible for the three different apoE isoproteins. Adapted from Alzheimer Research Forum (98) and Kim et al. 2014 (102).
Other potential genetic risk factors

In a Spanish study comprising 112 NPH patients and 124 controls, the polymorphism of the angiotensin I converting enzyme (ACE) was investigated (109). ACE codes a significant enzyme of the renin–angiotensin–aldosterone system, which is a major contributor to blood pressure regulation (110). The polymorphism of the ACE gene is based on insertion (I) and deletion (D) alleles, leading to three different genotypes: I/I, D/D, I/D, of which, in particular, the D/D genotype has been associated with cardiovascular diseases (110). The study found no differences in the distribution of the alleles between patients with NPH and controls, however, the D/D and I/D genotypes showed a weaker response to shunting compared to the I/I genotype (109). There are no other publications in the literature regarding the polymorphism of ACE in NPH.

In a small study in Japan, a segmental copy number loss of the Scm-like with four MBT domains 1 gene (SFMBT1) was observed in four of the eight cases with features of iNPH on MRI, while in ten control subjects, no such finding was reported (111). Due to the small number of subjects and unusual setting, the finding requires further study with a larger cohort with diagnosed iNPH patients and healthy controls. If the finding is replicated in a larger setting, it may shed light on the pathogenesis of iNPH.

2.6 DIAGNOSTICS OF INPH

2.6.1 Diagnostic criteria

Evidence-based international guidelines for the diagnosis of iNPH were published by an independent study group in 2005 (7). Based on signs and symptoms (see 2.2 Clinical features), brain imaging, and physiological tests, iNPH is classified into unlikely, possible, and probable categories according to the certainty of the diagnosis (Table 2) (7).
Table 2. Classification of idiopathic normal pressure hydrocephalus (iNPH) into probable, possible, and unlikely categories according to the international diagnostic guidelines. Adapted from Relkin et al. 2005 (7).

**Probable iNPH**
The diagnosis of *Probable iNPH* is based on clinical history, brain imaging, physical findings, and physiological criteria.

**I. History**
Reported symptoms should be corroborated by an informant familiar with the patient’s premorbid and current condition, and must include
   a. Insidious onset (versus acute)
   b. Origin after age 40 yr
   c. A minimum duration of at least 3 to 6 mo
   d. No evidence of an antecedent event such as head trauma, intracerebral hemorrhage, meningitis, or other known causes of secondary hydrocephalus
   e. Progression over time
   f. No other neurological, psychiatric, or general medical conditions that are sufficient to explain the presenting symptoms

**II. Brain imaging**
A brain imaging study (CT or MRI) performed after onset of symptoms must show evidence of
   a. Ventricular enlargement not entirely attributable to cerebral atrophy or congenital enlargement (Evans’ index >0.3 or comparable measure)
   b. No macroscopic obstruction to CSF flow
   c. At least one of the following supportive features
      1. Enlargement of the temporal horns of the lateral ventricles not entirely attributable to hippocampus atrophy
      2. Callosal angle of 40 degrees or more
      3. Evidence of altered brain water content, including periventricular signal changes on CT and MRI not attributable to microvascular ischemic changes or demyelination
      4. An aqueductal or fourth ventricular flow void on MRI

Other brain imaging findings may be supportive of an iNPH diagnosis but are not required for a probable designation
   1. A brain imaging study performed before onset of symptoms showing smaller ventricular size or without evidence of hydrocephalus
   2. Radionuclide cisternogram showing delayed clearance of radiotracer over the cerebral convexities after 48–72h
   3. Cine MRI study or other technique showing increased ventricular flow rate
   4. A SPECT-acetazolamide challenge showing decreased periventricular perfusion that is not altered by acetazolamide

**III. Clinical**
By classic definitions, findings of gait/balance disturbance must be present, plus at least one other area of impairment in cognition, urinary symptoms, or both.

With respect to gait/balance, at least two of the following should be present and not be entirely attributable to other conditions
   a. Decreased step height
   b. Decreased step length
   c. Decreased cadence (speed of walking)
   d. Increased trunk sway during walking
   e. Widened standing base
   f. Toes turned outward on walking
   g. Retropulsion (spontaneous or provoked)
   h. *En bloc* turning (turning requiring three or more steps for 180 degrees)
   i. Impaired walking balance, as evidenced by two or more corrections out of eight steps on tandem gait testing
With respect to cognition, there must be documented impairment (adjusted for age and educational attainment) and/or decrease in performance on a cognitive screening instrument (such as the Minimental State examination), or evidence of at least two of the following on examination that is not fully attributable to other conditions:

- Psychomotor slowing (increased response latency)
- Decreased fine motor speed
- Decreased fine motor accuracy
- Difficulty dividing or maintaining attention
- Impaired recall, especially for recent events
- Executive dysfunction, such as impairment in multistep procedures, working memory, formulation of abstractions/similarities, insight
- Behavioral or personality changes

To document symptoms in the domain of urinary continence, either one of the following should be present:

- Episodic or persistent urinary incontinence not attributable to primary urological disorders
- Persistent urinary incontinence
- Urinary and fecal incontinence

Or any two of the following should be present:

- Urinary urgency as defined by frequent perception of a pressing need to void
- Urinary frequency as defined by more than six voiding episodes in an average 12-hour period despite normal fluid intake
- Nocturia as defined by the need to urinate more than two times in an average night

### IV. Physiological

CSF opening pressure in the range of 5–18 mmHg (or 70–245 mmH₂O) as determined by a lumbar puncture or a comparable procedure. Appropriately measured pressures that are significantly higher or lower than this range are not consistent with a probable iNPH diagnosis.

### Possible iNPH

A diagnosis of Possible iNPH is based on historical, brain imaging, and clinical and physiological criteria.

#### I. History

Reported symptoms may

- Have a subacute or indeterminate mode of onset
- Begin at any age after childhood
- May have less than 3 mo or indeterminate duration
- May follow events such as mild head trauma, remote history of intracerebral hemorrhage, or childhood and adolescent meningitis or other conditions that in the judgment of the clinician are not likely to be causally related
- Coexist with other neurological, psychiatric, or general medical disorders but in the judgment of the clinician not be entirely attributable to these conditions
- Be nonprogressive or not clearly progressive

#### II. Brain imaging

Ventricular enlargement consistent with hydrocephalus but associated with any of the following:

- Evidence of cerebral atrophy of sufficient severity to potentially explain ventricular size
- Structural lesions that may influence ventricular size
Preceding the international diagnostic criteria, a set of guidelines were introduced in Japan in 2004 (112, 113). Originally, the Japanese guidelines followed the same scheme of diagnosis and the categories of possible and probable iNPH, but added a category of “definite iNPH”, if a positive response to shunting was observed (113). However, in the revision of the guidelines in 2012, a novel subgroup of iNPH with disproportionately enlarged subarachnoid space hydrocephalus (DESH) findings in the MRI, which negates the need for additional physiological tests in these patients, was included in the guidelines (27).

2.6.2 Neuroradiology
Historically, hydrocephalus was visualized with ventriculography (114) and more commonly with pneumoencephalography (115), where air injected to the CSF space created a contrast between the air-filled ventricles and the solid brain parenchyma. This was the golden standard of ventricular imaging until cross-sectional imaging modalities were introduced (116). In the anatomical cross-sectional imaging studies of the brain, e.g. CT and MRI, the contrast between the CSF and brain parenchyma is high and the ventricular size and the potential cause of the CSF flow obstruction can be readily assessed noninvasively (116).

The pivotal neuroradiological finding in iNPH is the enlargement of the brain ventricles without the presence of a causative factor such as cerebral atrophy, congenital enlargement, or macroscopic obstruction of the CSF flow (7). Other anatomical findings of iNPH seen in radiographic imaging include enlarged temporal horns (not attributable to hippocampus atrophy) and lateral sulci, callosal angle of 40 degrees or more, periventricular signal changes suggestive of altered water content of the brain, and tight subarachnoid space at high convexity (Table 2) (7, 27). A common method of ventricular size quantification is the calculation of Evans’ index by dividing the maximal horizontal width of the frontal horns by the maximal width of the inner skull at the same level (Figure 5) (117).

Table 2 Continued.

<table>
<thead>
<tr>
<th>III. Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms of either</td>
</tr>
<tr>
<td>a. Incontinence and/or cognitive impairment in the absence of an observable gait or balance disturbance</td>
</tr>
<tr>
<td>b. Gait disturbance or dementia alone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IV. Physiological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening pressure measurement not available or pressure outside the range required for probable iNPH</td>
</tr>
</tbody>
</table>

**Unlikely iNPH**

1. No evidence of ventriculomegaly
2. Signs of increased intracranial pressure such as papilledema
3. No component of the clinical triad of iNPH is present
4. Symptoms explained by other causes (e.g. spinal stenosis)

CT = computed tomography; MRI = magnetic resonance imaging; CSF = cerebrospinal fluid; SPECT = single-photon emission computed tomography

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2.6.3 Neuropsychology

According to the international diagnostic criteria, at least two of the following findings must be present in neuropsychological examination, if cognitive deterioration is attributed to iNPH: psychomotor slowing (increased response latency); decreased fine motor speed; decreased fine motor accuracy; difficulty dividing or maintaining attention; impaired recall, especially for recent events; executive dysfunction, such as impairment in multistep procedures, working memory, formulation of abstractions/similarities, insight; and behavioral or personality changes (Table 2) (7).

Various cognitive tests, including Minimental State examination (118) for screening and more comprehensive neuropsychological test batteries, are utilized to differentiate iNPH-related cognitive deterioration from other diseases causing cognitive impairment (see 2.6.6 Differential diagnosis and 2.7 Comorbidities). Wechsler Memory Scale logical memory subtest, Rey Auditory Verbal Learning Test, Trail Making B test, Rey-Osterrieth Complex Figure test, Line-Tracing test, Alzheimer’s Disease Assessment Scale, Wechsler Adult Intelligence Scale-Revised test, Stroop tests, and Grooved Pegboard test have been reported to be useful in the diagnostics and prognostication of iNPH (36, 38–41).

2.6.4 Tests of CSF circulation dynamics

In probable iNPH the CSF opening pressure, as measured by a lumbar puncture or comparable method, must be within 5–18 mmHg (70–245 mmH₂O) (Table 2) (7). Other supporting findings for iNPH in examinations of CSF physiology include sporadic rises in the CSF pressure in a direct ICP monitoring (67–69, 87, 119) and a low CSF outflow conductance in various infusion tests (119, 120).

The predictive value of CSF removal via a lumbar puncture was first noted by Hakim (1, 2). Lumbar CSF bolus removal (tap test) and external lumbar drainage (ELD) reduce the CSF pressure and absorption (essentially creating a temporary shunt) by removing 40–50 ml and 300–720 ml of CSF, respectively (119, 121, 122). The specificity of the tap test is considered to be high (72–100%), but sensitivity low (26–62%) (119). In contrast, ELD is both sensitive (50–100%) and specific (60–100%), but requires hospitalization and the rate of complications is higher compared to the tap test (119).

2.6.5 Diagnosis of iNPH post mortem

There are no identified pathognomonic signs of iNPH in the autopsy. On gross examination of

*Figure 5. Axial magnetic resonance imaging (MRI) scan demonstrating calculation of Evans’ index by dividing the maximal horizontal width of the frontal horns (a) by the maximal width of the inner skull (b). Evans’ index >0.3 is suggestive of hydrocephalus including idiopathic normal pressure hydrocephalus (iNPH) (7).*
the brain, dilated brain ventricles and possible fibrous thickening of the leptomeninges have been reported (143). Microscopic examination may reveal gaps in the ependymal lining, gliosis of the periventricular region, and ischaemic lesions in the deep white matter (143, 144).

2.6.6 Differential diagnosis

Alzheimer’s disease (AD) was first described in 1907 by Alois Alzheimer (145). Characteristic neuropathological findings include cerebral Aβ plaques and neurofibrillary tangles composed of hyperphosphorylated tau (HPT) aggregates in elderly patients with cognitive deterioration (146, 147). According to the amyloid cascade hypothesis, molecular and structural pathological changes precede the symptoms and clinical manifestation of the disease by decades (Figure 6) (148, 149). Common methods to evaluate the presence of accumulated amyloid in live persons include CSF sampling (see 2.6.7 CSF biomarkers as potential future diagnostic tools), positron emission tomography (PET) imaging (utilizing the $^{11}$C-labelled Pittsburgh compound B (150), $[^{18}$F]Flutemetamol (151), or $[^{18}$F]Florbetaben (152)) or rarely, brain biopsy (87, 153). Additionally, systemic inflammation and neuroinflammation may contribute to the pathogenesis of AD (154).

In a neuroradiological study, medial temporal lobe atrophy is the central finding in AD, and while AD patients may show ventricular enlargement, concomitant cortical atrophy is usually present in such cases (155–157). In AD, difficulties in learning and impairment of episodic memory or other cognitive domains such as visuospatial or linguistic skills are usually the early symptoms, whereas in iNPH, psychomotor slowing is typically present (7, 88, 158). In the progression of AD, extrapyramidal signs and symptoms may develop, however, typically in the more advanced stage of the disease, stance is usually narrower compared to iNPH (159).

Vascular cognitive impairment (VCI) is an umbrella term, which includes a spectrum of cerebrovascular diseases with cognitive symptoms from early mild cognitive impairment to late-stage dementia, and often presents in conjunction with AD in the elderly (160, 161). VCI can occur after stroke (cortical type) or due to ischaemia in the white matter (subcortical type), multiple small lacunae infarcts, or infarct at a strategic site (160). Subcortical vascular degeneration with or without dementia is the most important subtype of VCI regarding the differential diagnosis of iNPH, as the

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**Figure 6.** Biomarkers and progression of mild cognitive impairment (MCI) to Alzheimer’s disease (AD). AD-related pathology occurs years before manifestation of clinical symptoms. Adapted from Jack et al. 2010 (149).
gait, cognitive, and urinary symptoms bear a resemblance to iNPH (7, 160–162). In neuroimaging, ischaemic deep white matter lesions are the hallmark findings of subcortical VCI (160–162), however, such findings are also frequent in iNPH (9, 76, 82, 163–167). Gait disturbances and other iNPH-like symptoms may occur after stroke as well (7).

Dementia with Lewy bodies (DLB) and Parkinson’s disease (PD) with (PDD) or without dementia share the disturbance of α-synuclein metabolism and formation of Lewy bodies, ultimately leading to neurodegeneration (168). Clinically, DLB is characterized by neuropsychiatric symptoms, such as hallucinations and psychoses; adverse reaction to neuroleptic drugs; autonomic symptoms; REM-sleep disturbances; and cognitive impairment, which includes fluctuations in attention, executive function, visuospatial function, language function, memory, and behavior (168). The majority of patients with DLB eventually develop extrapyramidal motor disorder defined by bradykinesia, gait disorder, and postural disturbances without major tremor (168). However, patients with PDD develop motor deficits and a unilateral tremor years before clear cognitive deterioration occurs (168). Differential diagnosis between Lewy body disorders (PD, PDD, DLB) and iNPH is based on differences between clinical features (2.2 Clinical features) and radiological findings of iNPH that are not associated with Lewy body disorders (2.6.2 Neuroradiology).

Common diseases that may present with one or more of the iNPH symptoms (gait disorder, cognitive impairment, urinary incontinence) include frontotemporal dementia, traumatic brain injury, brain tumors, other hydrocephalic disorders, depression, spinal stenosis, and primary urological disorders (7).

2.6.7 CSF biomarkers as potential future diagnostic tools
CSF biomarkers are not included in the international diagnostic guidelines of iNPH. However, low CSF levels of amyloid beta 42 (Aβ42) (123) and increased levels of HPτ 181 and total tau (124), are supportive findings in the diagnostics of AD (25) and are helpful in differential diagnoses between AD and iNPH (26). Additionally, elevation of CSF tau has been associated with several other neurodegenerative disorders (125). Aβ is formed by the proteolytic cleavage of amyloid precursor protein (APP) (126). The cleavage of APP by the amyloidogenic β-secretase pathway produces additionally soluble APP beta (sAPPβ), while the α-secretase pathway precludes the formation of Aβ and results in the introduction of soluble APP alpha (sAPPα) (126). Lower CSF levels of both sAPP isoforms have been reported in iNPH compared to healthy individuals and AD (127–129), and subsequent increase in the ventricular CSF levels of sAPP isoforms after shunt surgery was noted in a single paper (129). In addition, sAPPα has shown a potential prognostic value in shunted iNPH patients (128).

Several other CSF biomarkers have been studied in iNPH, but none of them have become a routine diagnostic tool (130). Findings of proinflammatory cytokines in the CSF of iNPH patients have been rather contradictory (129, 131–136). In contrast, elevated levels of neurofilament light (NFL) protein in iNPH and secondary NPH have been reported consistently in several publications (129, 137–140). However, as NFL reflects axonal damage and neuronal loss, higher NFL levels have been reported in various other dementing disorders including AD and VCI compared to healthy individuals (141). A single article reported abnormal CSF transferrin-1/transferrin-2 isoform (Tf-1/Tf-2) ratios in patients with iNPH compared to healthy controls and AD patients (142).

2.7 COMORBIDITIES IN INPH

2.7.1 Degenerative brain disease
While AD is important in the differential diagnostics of iNPH (2.6.6.1 Alzheimer’s disease), these two diseases frequently coexist as iNPH-AD (9, 88). In a single study, iNPH-AD was reported in 12% of shunt-responding iNPH patients and iNPH-VCI in 5% (88). AD-related findings have been reported in 62% of NPH patients (pooled data from autopsy studies) on neuropathological examination (144, 169). In addition, Aβ plaques together with neurofibrillary tangles have been present in 23% of the living NPH patients (pooled data) in studies utilizing brain biopsy (144, 170, 171). Moreover, similar findings have been reported in CSF and PET studies (26, 150, 151). Current understanding indicates that AD pathology in iNPH patients is associated with a worse outcome after shunt surgery, but does not affect survival (9, 87, 88, 153, 170, 172–174). Nevertheless, a panel of
experts of the International Society for Hydrocephalus and Cerebrospinal Fluid Disorders recommends shunt treatment in patients with mixed iNPH-AD findings, however, the cognitive outcome in particular may be less satisfactory compared to typical iNPH patients (9). The common concurrence of iNPH and AD has lead some researchers to suggest common pathological pathways for the two diseases (83) (see 2.4.2 Pathophysiological theories). Even if no AD-related pathological findings are observed in the brain biopsy and shunt surgery is successful, in some persons with iNPH a clinical ‘hydrocephalic’ dementia has been diagnosed (88).

2.7.2 Vascular disease and type 2 diabetes mellitus
The burden of vascular risk factors (Table 3) increases an individual’s risk of developing vascular diseases such as coronary heart disease and potentially life-threatening complications (e.g. myocardial infarction) (175). A few studies have reported increased frequency of various vascular risk factors in iNPH patients (176–178). In particular, the prevalence of systemic arterial hypertension has been reported to be higher in iNPH (42–88%) compared to the age-matched hospital or population-based controls in several papers (8, 9, 176–180). Interestingly, one study reported a better cognitive response to shunt surgery in iNPH patients without a diagnosis of hypertension compared to hypertensive patients (180).

Table 3. List of cardiovascular risk factors. Adapted from Mancia et al. 2013 (175).

<table>
<thead>
<tr>
<th>Risk Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Age (men ≥55 years; women ≥65 years)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
</tr>
<tr>
<td>Total cholesterol &gt;4.9 mmol/L, and/or</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol &gt;3.0 mmol/L, and/or</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol: men &lt;1.0 mmol/L, women &lt;1.2 mmol/L, and/or</td>
</tr>
<tr>
<td>Triglycerides &gt;1.7 mmol/L</td>
</tr>
<tr>
<td>Hyperglycaemia</td>
</tr>
<tr>
<td>Abnormal fasting plasma glucose</td>
</tr>
<tr>
<td>Abnormal glucose tolerance test</td>
</tr>
<tr>
<td>Abdominal obesity (waist circumference men ≥102 cm; women ≥88 cm) (in Caucasians)</td>
</tr>
<tr>
<td>Family history of premature cardiovascular disease (men aged &lt;55 years; women aged &lt;65 years)</td>
</tr>
</tbody>
</table>

The prevalence of cardiovascular disease has been reported to be high in patients with iNPH (8, 176–178). In addition, deep white matter lesions in brain imaging and a diagnosed comorbid cerebrovascular disease are common in iNPH (8, 9, 76, 82, 163–166, 177, 181–183).

Type 2 diabetes mellitus (T2DM) is frequently associated with vascular diseases (184) and the reported relative prevalence of T2DM in iNPH is high (16–52%) (8, 176–178, 185). Although the association of vascular disease and iNPH is well-established, the underlying pathophysiology is currently not understood (9) (see 2.4.2 Pathophysiological theories).

2.7.3 Other comorbidities
A single paper reported an overrepresentation of glaucoma in 72 patients with iNPH compared to 72 age-matched non-iNPH hydrocephalic controls (18% vs. 5.6%, p = 0.02) (186). The authors hypothesized that similar mechanisms could be responsible for the normotensive glaucomatous optic neuropathy and axonal injury in iNPH (186). Interestingly, shunt treatment may cause progression of glaucoma due to a decrease of CSF pressure, which in turn increases translaminar pressure gradient of the optic nerve (187). Other potential comorbidities that can cause or worsen iNPH-
like symptoms include lumbar and cervical spinal stenosis, osteoarthrosis, and primary urological diseases (9).

2.8 TREATMENT OF INPH

The treatment of iNPH by CSF shunt surgery has been established since the first description of the disease (2, 3). A shunt is an implantable device that comprises of a proximal catheter, a one-way valve, and a distal catheter creating an alternative pathway for the CSF drainage. Shunt systems are categorized by the placement of the catheters: a ventriculoperitoneal (VPS) (188) and a ventriculoatrial (VAS) (189) shunt drain CSF from a lateral ventricle of the brain to the peritoneal cavity or to the right atrium of the heart, respectively; while a lumboperitoneal shunt (LPS) (190) drains CSF from the lumbar CSF space to the peritoneal cavity.

There are currently no randomized controlled trials comparing shunt surgery with a closed shunt or sham surgery (no shunt), and thus the level I evidence on the efficacy of shunt surgery in iNPH is lacking (191). The highest level of evidence to date is presented by a Japanese open-label randomized trial that compared immediate (<1 month after randomization) and postponed (3 months) LPS surgery and reported a 65% improvement in the first group and 5% in the latter at three months ($p < 0.0001$) concluding that LPS may be beneficial in the treatment of iNPH (192). While there is no level I evidence on shunt surgery, several studies have reported a beneficial outcome after shunt surgery in most selected patients (see 2.9.2 Outcome of treatment) and VPS surgery remains the recommended treatment for iNPH (193).

An alternative management for hydrocephalus is the endoscopic third ventriculostomy (ETV) that is mainly utilized for the obstructive hydrocephalus (194, 195). In ETV, the floor of the third ventricle is perforated endoscopically creating an alternative tract from the ventricular CSF system to the skull base subarachnoid space (194, 195). The role of ETV in the nonobstructive hydrocephalus, such as iNPH, is controversial, since although success rates similar to shunting have been reported (196), an open-label, noncrossover, randomized trial found VPS to be superior compared to ETV in iNPH (197). A Cochrane Review found no high-quality evidence to determine the effectiveness of ETV in patients with iNPH (198).

There is currently no established pharmacological treatment for iNPH. However, acetazolamide (ACZ), a carbonic anhydrase inhibitor that reduces CSF production, is widely used in glaucoma (199), idiopathic intracranial hypertension (200, 201), and for the prophylaxis of acute mountain sickness (202). In iNPH, ACZ has been subject to a single case report and two small studies that have demonstrated a potential to alleviate gait symptoms and to reduce white matter hyperintensities (203–205). However, as these studies have a very small number of patients ($n = 1–15$) and no control group, there is currently no scientific evidence for the efficacy of ACZ in iNPH.

2.9 PROGNOSIS OF INPH

2.9.1 Natural course

The natural course of iNPH in unshunted patients is unclear (206). The studies that have included unshunted iNPH patients, have shown most patients to deteriorate and some to remain stable (206–213). However, all the cited studies that included unshunted iNPH patients suffer from selection bias, as no randomization was undertaken. A double-blind, randomized trial with a closed shunt or sham surgery as a control would give insight on the natural course of iNPH in unselected patients. A trial has been published at a small scale ($n = 14$) with iNPH patients with findings of comorbid subcortical vascular dementia and negative findings in CSF infusion studies, suggesting that shunt treatment is beneficial to these patients (183).

2.9.2 Outcome of treatment

A substantial number of papers studying the outcome of shunt treatment in iNPH have been published. A systematic review comprising 64 studies and 3,063 patients with iNPH reported an average improvement of 71% in short-term (3–12 months after surgery) and 65% in long-term (at least more than 3 years after surgery) with an average of 1% mortality and complications rate of 10.4% (214). A better outcome (82%) and lower mortality (0.2%) were noted in the studies published in
2006 or later compared to earlier papers (214). The most common complications of shunt treatment include a subdural haemorrhage or effusion (6.3%), infections (3%), and a new onset of seizures (0.7%) (214). In ETV, higher mortality (3.2% vs. 0.5%) and short-term complication rates (17.9% vs. 11.8%) have been reported (215).

2.9.3 Mortality and causes of death
High frequency of vascular disease comorbidity seems to affect the mortality and causes of death in patients with iNPH – cerebrovascular and cardiovascular disease comprise 52% of the causes of death in iNPH (pooled data from eight studies including a total of 110 deceased iNPH patients) (177, 216–222). Following vascular diseases, the most common causes of death in patients with iNPH are injury (15%) and malignant neoplasms (11%) (177, 216–222). Interestingly, dementia or iNPH as a cause of death is rare (0.1%) (177, 216–222). For a full review of the literature regarding mortality and causes of death in iNPH, see Table 4. No causes of death were provided for control populations in the cited studies, however, ischaemic heart disease and stroke are the most common causes of death in high income countries (223).
Table 4. Mortality and causes of death in idiopathic normal pressure hydrocephalus (iNPH) in literature.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients, n</th>
<th>Follow-up time, mo</th>
<th>Deaths, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenberg et al. 1977</td>
<td>73 iNPH</td>
<td>3–66(^a)</td>
<td>29 (39.7)</td>
</tr>
<tr>
<td>(216)</td>
<td></td>
<td>9.7(^b) (series 1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.7(^b) (series 2)</td>
<td></td>
</tr>
<tr>
<td>Black 1980 (217)</td>
<td>62 iNPH</td>
<td>2.25–75(^a)</td>
<td>5 (8.1)</td>
</tr>
<tr>
<td>Raftopoulos et al. 1996 (218)</td>
<td>23 iNPH</td>
<td>9–68(^a)</td>
<td>13 (56.5)</td>
</tr>
<tr>
<td>Boon et al. 1998 (219)</td>
<td>101 iNPH</td>
<td>&lt;1–12(^a)</td>
<td>16 (15.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.9±3.1(^c) (group 1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.7±3.2(^c) (group 2)</td>
<td></td>
</tr>
<tr>
<td>Malm et al. 2000 (220)</td>
<td>42 iNPH</td>
<td>&lt;3–36(^a)</td>
<td>12 (28.6)</td>
</tr>
<tr>
<td>Aygok et al. 2005 (258)</td>
<td>50 iNPH</td>
<td>36</td>
<td>4 (8.0)</td>
</tr>
<tr>
<td>Spagnoli et al. 2006 (221)</td>
<td>66 iNPH</td>
<td>4–96(^a)</td>
<td>16 (24.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52±24.8(^e)</td>
<td></td>
</tr>
<tr>
<td>Tisell et al. 2006 (259)</td>
<td>109 total 38 iNPH</td>
<td>&lt;3–74(^a)</td>
<td>29 (28.7) total</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.4(^d)</td>
<td>14 (36.8) iNPH</td>
</tr>
<tr>
<td>Kahlon et al. 2007 (260)</td>
<td>75 total 58 iNPH</td>
<td>&gt;6–91(^a)</td>
<td>28 (37.3) total</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.1±4.6(^c) (short-term)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>66±16.2(^c) (long-term)</td>
<td></td>
</tr>
<tr>
<td>Mirzayan et al. 2010 (177)</td>
<td>51 iNPH</td>
<td>18.8±16.6(^c) (short-term)</td>
<td>29 (56.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80.9±51.6(^c) (long-term)</td>
<td></td>
</tr>
<tr>
<td>Sprung et al. 2010 (261)</td>
<td>165 total 69 iNPH</td>
<td>12</td>
<td>9 (5.5) total</td>
</tr>
<tr>
<td>Lundkvist et al. 2011 (262)</td>
<td>72 iNPH</td>
<td>3–79 (^e)</td>
<td>7 (9.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35(^d)</td>
<td></td>
</tr>
<tr>
<td>Leinonen et al. 2012 (170)</td>
<td>468 NPH</td>
<td>0–204(^a)</td>
<td>267 (57.1)</td>
</tr>
<tr>
<td>Stranjalis et al. 2012 (263)</td>
<td>238 iNPH</td>
<td>Not specified</td>
<td>4 (1.7)</td>
</tr>
<tr>
<td>Gölz et al. 2014 (222)</td>
<td>147 iNPH</td>
<td>72 (for 61 patients, others died or lost to follow-up)</td>
<td>69 (46.9)</td>
</tr>
</tbody>
</table>

\(^a\)Range  
\(^b\)Mean  
\(^c\)Mean±SD  
\(^d\)Median
### Cause of death

24 unrelated to shunt surgery, otherwise not specified. 5 patients died within one month of surgery: myocardial infarction (n = 1), stroke (n = 1), pneumonia (n = 1), perforated duodenal ulcer (n = 1), pulmonary embolism and uraemia (n = 1).

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>n = 1</td>
</tr>
<tr>
<td>Stroke</td>
<td>n = 3</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>n = 1</td>
</tr>
<tr>
<td>Perforated duodenal ulcer</td>
<td>n = 1</td>
</tr>
<tr>
<td>Pulmonary embolism and uraemia</td>
<td>n = 1</td>
</tr>
</tbody>
</table>

All within 3 months of surgery.

Stroke (n = 4), myocardial infarction (n = 3), digestive tract neoplasia (n = 1), ovarian neoplasia (n = 1), pneumonia (n = 1), septicemia (n = 1), struck by a car (n = 1), not specified (n = 1).

Pneumonia (n = 4), cardiac failure (n = 3), stroke (n = 3), mesenteric arterial thrombosis (n = 1), peritonitis (n = 1), head injury (n = 1), unknown (n = 3).

Intracerebral hemorrhage (n = 4), myocardial infarction (n = 2), abdominal cancer (n = 2), pulmonary complications following shunt surgery (n = 1), ruptured aortic aneurysm (n = 1), dementia (n = 1), complications following a subdural hematoma (n = 1).

Unrelated to shunt surgery, otherwise not specified.

Cardiac failure (n = 4), stroke (n = 3), pneumonia (n = 3), kidney cancer (n = 1), unknown (n = 5).

Heart disease (n = 7), malignancy (n = 7), infection (n = 7), stroke (n = 5), epileptic status (n = 1), choking (n = 1), unknown (n = 1). No separate data for the iNPH subgroup was provided.

Cardiovascular disease (n = 9), malignancy (n = 7), pneumonia (n = 3), septicaemia (n = 2), dementia (n = 2), pancreatitis (n = 1), unknown (n = 4). No separate data for the iNPH subgroup was provided.

Cerebral infarction (n = 12), cardiac failure (n = 7), cancer (n = 2), pneumonia (n = 2), acute respiratory distress syndrome (n = 1), pulmonary embolism (n = 1), renal failure (n = 1), unknown (n = 3).

Unspecified internal disease (n = 3), myocardial infarction (n = 2), pneumonia (n = 2), aortofemoral bypass operation (n = 1), stroke (n = 1). No separate data for the iNPH subgroup was provided.

Unrelated to shunt surgery, otherwise not specified.

Cardiovascular (n = 49), ischaemic stroke (n = 26), haemorrhagic stroke (n = 7), any dementia (n = 46), infection (n = 23), other (n = 77), data not available (n = 39). No separate data for the iNPH subgroup was provided.

Shunt-related death (n = 3), unrelated to shunt surgery (n = 1). Only hospital mortality reported.

Pneumonia (n = 7), cardiovascular diseases and heart attacks (n = 5), malignancy (n = 5), stroke (n = 3), multiple organ failure following liver dysfunction (n = 3), renal failure (n = 2), sepsis due to peritonitis (n = 1), acute paraplegia (n = 1), not specified (n = 42).
3 Aims of the study

The aim of this thesis was to investigate the general characteristics of a large cohort of patients from a defined geographical area with possible iNPH and to evaluate potential biomarkers.

The specific aims were:

1.) To determine incidence of iNPH in Middle and East Finnish populations (i).

2.) To assess comorbidities, mortality, and causes of death in patients with iNPH (ii).

3.) To study the effects of APOE genotypes in the diagnostics and prognostics in iNPH patients (iii).

4.) To evaluate a large panel of CSF biomarkers in iNPH and how they relate to brain biopsy findings (iv).

Roman numerals refer to original publications.
4 Incidence, comorbidities, and mortality in idiopathic normal pressure hydrocephalus

4.1 ABSTRACT

4.1.1 Objective
To investigate incidence, comorbidities, mortality, and causes of death in idiopathic normal pressure hydrocephalus (iNPH).

4.1.2 Methods
A cohort of 536 patients with possible NPH from a defined population with a median follow-up time of 5.1 years (range 0.04–19.9) was included in the study. Patients were evaluated by brain imaging and intraventricular pressure monitoring with a brain biopsy immunostained against amyloid-β and hyperphosphorylated tau. Hospital records were reviewed for vascular diseases and type 2 diabetes mellitus (T2DM). Death certificates and yearly population of the catchment area were obtained from national registries.

4.1.3 Results
A total of 283 patients had a clinical diagnosis of iNPH leading to a median annual incidence of 1.58 iNPH patients per 100,000 inhabitants (range 0.8–4.5). Alzheimer’s disease-related brain biopsy findings were less frequent in iNPH compared to the non-iNPH patients ($p<0.05$). An overrepresentation of hypertension (52% vs. 33%, $p<0.001$) and T2DM (23% vs. 13%, $p=0.002$) was noted in iNPH patients. Age (hazards ratio (HR) 1.04/year, 95% confidence interval (CI) 1.03–1.06, $p<0.001$) and T2DM (HR 1.63, 95% CI 1.23–2.16, $p<0.001$) increased the risk of death in the iNPH patients and in the total population. iNPH was associated with decreased risk of death (HR 0.63, 95% CI 0.50–0.78, $p<0.001$). The most frequent causes of death were cardiovascular and cerebrovascular disease. Dementia as a cause of death was more common in non-iNPH patients compared to iNPH (27% vs. 10%, $p<0.001$).

4.1.4 Conclusions
Hypertension and T2DM are common in iNPH and the latter causes excess mortality in the affected patients.

4.2 INTRODUCTION

Idiopathic normal pressure hydrocephalus (iNPH) is characterized by gait disturbance, cognitive decline, and urinary incontinence in the elderly with signs of communicating hydrocephalus in a neuroradiological study, while the cerebrospinal fluid (CSF) pressure is within normal range, and no predisposing factors such as subarachnoid hemorrhage are observed (2, 3). Despite the absence of elevated CSF pressure, the diversion of CSF with an implantable shunt device relieves and even reverses the symptoms in most patients (193).

iNPH is generally regarded as a rare disease with a reported incidence of 0.5–1.2 shunted iNPH patients per 100,000 inhabitants per year in hospital-based studies conducted in the Netherlands (10), Germany (11), Sweden (12), Norway (14), and the United States of America (15) (Table 1). In Japan, a hundredfold incidence (1.2 / 1000 inhabitants / year) of possible iNPH was reported in inhabitants aged 70 or older (48) (Table 1). The only true epidemiological population-based study of iNPH reported an incidence of at least 5.5 per 100,000 inhabitants per year in Norway indicating iNPH to be an underdiagnosed or undertreated disease (13) (Table 1). Interestingly, a single study
reported no diagnosed iNPH patients in a population-based dementia study of five years in the United States of America among 560 patients with nondegenerative and nonvascular dementia (49) (Table 1).

Arterial hypertension is the best documented concomitant disease with iNPH with a reported prevalence of 42–88% among iNPH patients (8, 9, 176–180). A single article noted a better cognitive outcome following shunt surgery in iNPH patients without hypertension compared to iNPH patients with diagnosed hypertension (180). Additionally, white matter vascular degeneration in brain imaging (8, 9, 76, 82, 163, 164, 177), cardiovascular disease (8, 176–178), vascular risk factors (176–178), and type 2 diabetes mellitus (T2DM) (8, 176–178) have been frequently associated with iNPH. It is currently unknown which mechanisms affect the concurrence of vascular disease and iNPH (9). A heavy burden of vascular disease reflects the mortality and causes of death in patients with iNPH: the most frequently reported causes of death are cerebrovascular disease and heart diseases (177, 216–222). For a summary of mortality and causes of death in iNPH, see Table 4.

Alzheimer’s disease (AD) is considered to be significant in the differential diagnostics of iNPH with cognitive symptoms, however, the two diseases are not exclusive, and in fact, frequently coexist as iNPH-AD (9). AD-related neuropathological findings have been reported in 62% of the NPH cases on autopsy (144, 169) and 34% of the living NPH patients studied with brain biopsy (144, 170). While pathological findings of AD in iNPH patients predict a worse cognitive outcome of shunt surgery, current expert opinion (9) does not recommend against shunting in iNPH-AD patients, as some benefit is still usually seen (87, 153, 170, 172, 173). Thus, shunt surgery in iNPH patients with a comorbid neurodegenerative disease should be considered individually depending on the disease severity and estimated benefits.

Objectives of the current study:
1. to determine the incidence of iNPH in Middle and Eastern Finland,
2. study the association between vascular diseases, T2DM, and iNPH,
3. assess the cumulative mortality and causes of death in iNPH.

4.3 METHODS

4.3.1 Kuopio NPH Registry and protocol
The patients of the current study were recruited from a defined geographical area in Middle and Eastern Finland, of which the sole serving acute and elective neurosurgical unit is the department of neurosurgery of the Kuopio University Hospital (KUH). There are four central hospitals (Central Finland Central Hospital, Mikkeli Central Hospital, Savonlinna Central Hospital, and North Karelia Central Hospital) in the KUH area with neurological units, which refer possible NPH patients to KUH Neurosurgery. The annual population of the KUH catchment area during the recruitment period was obtained from Statistics Finland (224).

Patients fulfilling the following criteria were further assessed in KUH Neurosurgery as possible NPH patients: (1) primary evaluation and examination by a neurologist; (2) one to three symptoms suggestive of NPH (gait disorder, cognitive decline, urinary incontinence); and (3) NPH-related brain imaging findings. The diagnostic workup protocol of KUH Neurosurgery for possible NPH included a clinical examination, computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain, and a 24h intraventricular pressure monitoring together with a frontal cortical brain biopsy. During the final year of the recruitment period, 13 patients underwent a tap test and an infusion study instead of intracranial pressure (ICP) monitoring, and the cortical brain biopsy was obtained during the shunt operation (225). Kuopio NPH Registry (www.uef.fi/nph) consists of all evaluated possible NPH patients from the KUH catchment population since 1993 and contains clinical baseline and follow-up data, other hospital diagnoses, medications, causes of death from national registries, and neuropathological findings.

4.3.2 Participants
All patients meeting the criteria above during the recruitment period (1993–2010) were initially enrolled to the study. Patients outside the current KUH catchment area and patients diagnosed with secondary NPH were excluded from the current study. Included patients were followed-up until
death or the end of December 2013. All patients or their proxies gave a written, informed consent prior to participation in the study.

4.3.3 Shunt treatment and shunt response
In iNPH patients, ICP criteria indicating shunt treatment were (1) a basal ICP pressure between 10 and 20 mmHg continuously during the 24h monitoring, or (2) the presence of A-waves or more than 30% B-waves, when basal pressure was between 5 and 10 mmHg (207). The shunt response was clinically evaluated at 2–3 months after surgery, and any subjective or objective improvement in the patient’s gait, memory, or urinary continence was graded as a positive shunt response (87).

4.3.4 Comorbidities and causes of death
Presence or absence of a diagnosed arterial hypertension, coronary artery disease, atrial fibrillation, chronic heart failure, and T2DM were reviewed from hospital records. Causes of death were obtained from national clinical registries.

4.3.5 Immunohistochemistry and histological evaluation of brain biopsy
A frontal cortex sample approximately 10 mm in length and 2 mm in diameter was obtained during the ICP monitoring or shunt operation. A hematoxylin-eosin staining revealed that in most samples both grey and white matter were represented. Seven micrometre thick sections were stained applying immunohistochemistry to visualize β-amyloid (Aβ) (6 clone 6F/3D) and hyperphosphorylated tau (HPτ) (clone AT8) as described earlier (87). Immunoreactivity for each antibody was assessed as present or absent.

4.3.6 Statistical analysis
For nominal variables (sex, leading symptom, immunoreactivity, comorbidities, causes of death), differences between groups were analyzed with the $\chi^2$ test or Fisher’s exact test, when applicable. The Mann–Whitney U test was utilized in continuous variables (age and follow-up time). In multiple comparisons, Bonferroni correction was used. Correlation of incidence was analyzed with Spearman’s correlation and linear regression. In survival analyses, Kaplan-Meier with log rank test and Cox proportional hazards model were applied.

IBM SPSS Statistics for Mac (version 22.0.0.0, IBM, Armonk, NY, USA) was used in the statistical analyses. The level of significance was set at $p < 0.05$.

4.3.7 Ethical issues
This study was approved by the Research Ethics Committee of the Northern Savo Hospital District, The Finnish National Supervisory Authority for Welfare and Health, and The Finnish Ministry of Social Affairs and Health.

4.4 RESULTS

4.4.1 Study population
All together 635 patients met the initial inclusion criteria for the study. A total of 99 patients were then excluded (40 patients outside of the current KUH catchment area, 59 patients with secondary NPH), which lead to the final study population of 536 patients with possible iNPH. Of the final study population, 283 patients had a clinical diagnosis of iNPH and 253 patients did not have iNPH. Overall, 269 of the iNPH patients were shunted with 229 patients (85%) showing a positive response. Patient characteristics of iNPH and non-iNPH patients are summarized in Table 5. Both groups were similar in age (median age at the time of ICP monitoring 73.3 vs. 73.5 years), however, there were more women in the iNPH patient group (57% vs. 46%, $p = 0.02$). Gait disorder was the most common leading symptom in the iNPH group, while cognitive decline was more frequent in the other group ($p < 0.001$).
<table>
<thead>
<tr>
<th></th>
<th>INPH</th>
<th>non-iNPH</th>
<th>p</th>
<th>post hoc p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>283</td>
<td>253</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) (median (range))</td>
<td>73.28 (43.49–87.86)</td>
<td>73.51 (29.57–86.13)</td>
<td>0.82⁺</td>
<td></td>
</tr>
<tr>
<td>Women (n (%))</td>
<td>161 (57)</td>
<td>117 (46)</td>
<td>0.015ᵇ</td>
<td></td>
</tr>
<tr>
<td>Follow-up time (years) (median (range))</td>
<td>5.56 (0.04–19.87)</td>
<td>4.83 (0.05–19.70)</td>
<td>0.019ᵃ</td>
<td></td>
</tr>
<tr>
<td><strong>Leading symptom (n (%))</strong></td>
<td>&lt;0.001ᵈ</td>
<td>&lt;0.001ᵇᵃ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gait impairment</td>
<td>127 (50)</td>
<td>50 (21)</td>
<td>&lt;0.001ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>61 (24)</td>
<td>88 (37)</td>
<td>0.01ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>67 (26)</td>
<td>103 (43)</td>
<td>&lt;0.001ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td><strong>Immunoreactivity (n (%))</strong></td>
<td>&lt;0.001ᵈ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ – HPτ –</td>
<td>169 (60)</td>
<td>109 (43)</td>
<td>&lt;0.001ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Aβ + HPτ –</td>
<td>86 (30)</td>
<td>99 (39)</td>
<td>0.14ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Aβ + HPτ +</td>
<td>24 (8)</td>
<td>41 (16)</td>
<td>0.03ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Aβ – HPτ +</td>
<td>3 (1)</td>
<td>2 (1)</td>
<td>0.99ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td><strong>Comorbidities (n (%))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>148 (52)</td>
<td>84 (33)</td>
<td>&lt;0.001ᵇ</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>53 (18)</td>
<td>61 (24)</td>
<td>0.14ᵇ</td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>20 (7)</td>
<td>9 (4)</td>
<td>0.09ᵇ</td>
<td></td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>18 (6)</td>
<td>25 (10)</td>
<td>0.15ᵇ</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>65 (23)</td>
<td>32 (13)ᵇ</td>
<td>0.002ᵇ</td>
<td></td>
</tr>
<tr>
<td><strong>Mortality (deaths) (n (%))</strong></td>
<td>144 (51)</td>
<td>181 (72)</td>
<td>&lt;0.001ᵇ</td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td>31 (22)</td>
<td>30 (17)</td>
<td>0.99ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>27 (19)</td>
<td>41 (23)</td>
<td>0.99ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Malignant neoplasm</td>
<td>18 (13)</td>
<td>16 (9)</td>
<td>0.99ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>11 (8)</td>
<td>16 (9)</td>
<td>0.99ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Injury</td>
<td>4 (3)</td>
<td>8 (4)</td>
<td>0.99ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>14 (10)</td>
<td>48 (27)</td>
<td>&lt;0.001ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>iNPH</td>
<td>13 (9)</td>
<td>0 (0)</td>
<td>&lt;0.001ᵇᵃ</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>25 (17)</td>
<td>22 (12)</td>
<td>0.99ᵇᵃ</td>
<td></td>
</tr>
</tbody>
</table>

⁺Mann-Whitney U test
ᵇFisher’s exact test
ᶜSufficient data for the determination of a leading symptom was unavailable in 28 INPH patients and 12 non-iNPH patients
ᵈχ² test
ᵉBonferroni corrected
ᵇBiopsy data was unavailable from 2 non-iNPH patients and 1 iNPH patient
ᶜPresence/absence of T2DM was unknown in three patients from the non-iNPH group
ᵈCause of death was not available in 1 iNPH patient
4.4.2 Catchment population and the incidence of iNPH

Catchment population of KUH Neurosurgery decreased from 875,184 to 842,918 inhabitants during the recruitment period (1993–2010) of the current study (Figure 7). In contrast, the number of inhabitants aged 70 or older increased constantly from 87,112 to 120,423 during the study period.

The cumulative incidence of iNPH ranged from 0.8 to 4.5 cases per 100,000 inhabitants per year with a mean incidence of 1.84 and standard deviation (SD) of 0.99 (Figure 7). Incidence correlated with the increasing proportion of inhabitants aged 70 or older in the population (Spearman’s $\rho = 0.77$, $p < 0.001$) and with the year of diagnosis (Spearman’s $\rho = 0.77$, $p < 0.001$). A linear regression analysis including the proportion of inhabitants aged 70 or older and the year of diagnosis resulted in correlation coefficient $R = 0.82$ and coefficient of determination $R^2 = 0.67$, $p < 0.001$. In the inhabitants aged 70 or older, the cumulative age-specific incidence of iNPH ranged from 7.1 to 31.6 with a mean incidence of 14.65 (SD 6.51).

4.4.3 Vascular disease and T2DM in iNPH

Hypertension was significantly more frequent in patients with a diagnosis of iNPH compared to non-iNPH patients with a prevalence of 52% in iNPH and 33% in non-iNPH ($p < 0.001$) (Table 5). Similar overrepresentation was noted in the frequency of T2DM (23% vs. 12%, $p = 0.002$) (Table 5). No differences were observed in other vascular diseases (Table 5).
4.4.4 Brain biopsy findings

Biopsy findings are summarized in Table 5. iNPH patients lacked more frequently Aβ and HPτ immunoreactivity ($p < 0.001$), whereas non-iNPH patients displayed Aβ+ HPτ+ more commonly ($p = 0.03$) (Table 5).

4.4.5 Mortality and causes of death in iNPH

During the follow-up 144 (51%) patients with iNPH and 181 (72%) patients with a final diagnosis other than iNPH died ($p < 0.001$) (Table 5). In iNPH, median estimated survival time was 9.0 years (95% confidence interval (CI) 7.6–10.5 years) and in non-iNPH patients 5.8 years (95% CI 4.9–6.7 years) ($p < 0.001$) (Figure 8).

![Kaplan-Meier survival plot](image)

**Figure 8.** Kaplan-Meier survival plot of 283 patients with idiopathic normal pressure hydrocephalus (iNPH) (blue line) and 253 non-iNPH patients (red line). Vertical tick-marks indicate censored cases.
The causes of death for both groups are summarized in Table 5 and Figure 9. The most common cause of death in patients with iNPH was heart disease (22%) and cerebrovascular disease (23%) for the non-iNPH patients. Dementia was a more frequent cause of death in non-iNPH patients compared to iNPH patients (27% vs. 10%, \(p < 0.001\)).

A Cox proportional hazards model was implemented to study the survival of the patients further (Figure 10). In the multivariable analysis, significant covariates were age (Hazards ratio (HR) 1.04/year, 95% CI 1.03–1.06, \(p < 0.001\)), chronic heart failure (HR 1.51, 95% CI 1.03–2.21, \(p = 0.04\)), T2DM (HR 1.63, 95% CI 1.23–2.16, \(p < 0.001\)), and iNPH (HR 0.63, 95% CI 0.50–0.78, \(p < 0.001\)) in the total population (Figure 10). In patients with iNPH (within-group analysis), significant covariates were age (HR 1.08/year, 95% CI 1.04–1.11, \(p < 0.001\)), atrial fibrillation (HR 2.27, 95% CI 1.20–4.29, \(p = 0.01\)), and T2DM (HR 1.71, 95% CI 1.15–2.54, \(p = 0.008\)).

Figure 9. Causes of death in 143 deceased idiopathic normal pressure hydrocephalus (iNPH) patients (lighter shade lower bars) and 185 non-iNPH patients (darker shade upper bars). \(P\)-values were determined using a Mann–Whitney U test.
<table>
<thead>
<tr>
<th>Covariate</th>
<th>n</th>
<th>HR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>275</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>254</td>
<td>1.18 (0.93–1.49)</td>
<td>0.18</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td>1.04 (1.03–1.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Immunoreactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ− HPτ−</td>
<td>276</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Aβ+ HPτ−</td>
<td>183</td>
<td>0.96 (0.74–1.23)</td>
<td>0.73</td>
</tr>
<tr>
<td>Aβ+ HPτ+</td>
<td>65</td>
<td>1.26 (0.88–1.81)</td>
<td>0.20</td>
</tr>
<tr>
<td>Aβ− HPτ+</td>
<td>5</td>
<td>1.54 (0.56–4.26)</td>
<td>0.40</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>111</td>
<td>1.12 (0.86–1.46)</td>
<td>0.41</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>29</td>
<td>1.33 (0.81–2.18)</td>
<td>0.27</td>
</tr>
<tr>
<td>Other arrhythmia</td>
<td>25</td>
<td>1.33 (0.83–2.12)</td>
<td>0.24</td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>42</td>
<td>1.51 (1.03–2.21)</td>
<td>0.04</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>232</td>
<td>1.00 (0.79–1.27)</td>
<td>0.98</td>
</tr>
<tr>
<td>T2DM</td>
<td>97</td>
<td>1.63 (1.23–2.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>iNPH</td>
<td>282</td>
<td>0.63 (0.50–0.78)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Figure 10.* A multivariable Cox proportional hazards model of 529 patients with suspected normal pressure hydrocephalus (NPH) and a forest plot of adjusted hazards ratios (HR) with 95% confidence intervals (CI). 7 patients were excluded from the analysis due to insufficient patient records. Aβ = amyloid-β; HPτ = hyperphosphorylated tau; T2DM = type 2 diabetes mellitus; iNPH = idiopathic normal pressure hydrocephalus.
4.5 DISCUSSION

This is currently the largest study to evaluate incidence, comorbidities, mortality and causes of death in iNPH. The main finding was the demonstration of a major vascular and diabetic comorbidity in patients with iNPH and how it reflects the mortality and causes of death in iNPH in a large cohort from a defined geographical area during a long-term follow-up.

Mean incidence of iNPH in the study population was 1.84 / 100,000 / year (SD 0.99) with a markedly higher age-specific incidence in the elderly (70 or older) of 14.65 (SD 6.51). The incidence of iNPH increased during the recruitment period (1993–2010) both in the total population and in the elderly, possibly due to better recognition of the disease among general practitioners and in specialist care. The incidence figures we found are higher than previously reported in hospital-based studies (10–12, 14, 15) but lower than in population-based studies (13, 48). It is unclear whether iNPH is more frequent in Finland compared to other countries or that the observed differences are due to dissimilarities in the study designs.

As expected, arterial hypertension was frequent in iNPH patients with a prevalence of 52% among patients with a diagnosis of iNPH (Table 5), which is in line with the reported prevalences in previous studies (42–88%) (8, 9, 176–180). According to the multivariable model, a diagnosis of hypertension did not affect mortality (Figure 10).

Additionally, T2DM was twice more frequent in patients with iNPH compared to non-iNPH patients of the cohort (23% vs. 13%, p = 0.002) (Table 5), which is a confirmatory finding for earlier studies (8, 176–178). Interestingly, although T2DM increased the risk of death in the total population (HR 1.63, 95% CI 1.23–2.16, p < 0.001) and in patients with iNPH (HR 1.71, 95% CI 1.15–2.54, p = 0.008), the overall mortality of iNPH patients was lower and thus iNPH was associated with a decreased risk of death compared to non-iNPH patients in the cohort (HR 0.63, 95% CI 0.50–0.78, p <0.001) (Figure 10). In future studies, the relationship of vascular disease and iNPH should be explored more: is the high vascular comorbidity in iNPH due to shared genetic or environmental factors, or are the diseases interlinked with the same pathological processes?

As reported earlier by our group (87, 88, 170), AD-related brain biopsy findings (Aβ+ HPτ+) were reported in 8.5% of the patients with iNPH. A tendency towards higher mortality was noted in patients with Aβ+ HPτ+, possibly reflecting AD-associated mortality (Figure 10).

In iNPH, leading causes of death were heart disease (22%) and cerebrovascular disease (18%), which is similar to pooled data from earlier reports (24% and 28%, respectively) (177, 216–222). Conversely, in our cohort, dementia was listed as a main cause of death in 10% of patients with iNPH, however, in previous studies (177, 216–222) this figure was only 0.1% (pooled data). Infection and injury were more frequently listed as a cause of death in earlier studies (20% and 15%) compared to our findings (8% and 3%) (177, 216–222). The frequency of malignant neoplasm as a cause of death was similar in our cohort (10.9% vs. 12.8%). Importantly, none of the cited studies reported iNPH as a cause death, however, in our cohort, iNPH was listed as a main cause of death in 9%. The observed differences in the causes of death are probably explained by the differences in listing and determining causes of death in different countries rather than actual pathobiological processes. Of the thirteen patients with iNPH listed as a main cause of death, one patient died of complications following ICP measurement (in the autopsy, immediate cause of death was aspiration pneumonia), and the twelve other patients had a chain of events unrelated to ICP measurement or shunt surgery. However, as no autopsy was performed in these patients, the true final cause of death is not certain.

Strengths of the current study included: a large cohort evaluated by cortical brain biopsy from a defined catchment area, a long-term follow-up, and systematically kept medical records for comprehensive retrospective review.

The retrospective nature of the study caused some limitations: blood pressure measurements or blood glucose tests (a fasting glucose test, a glucose tolerance test, or haemoglobin A1c (HbA1c)) were not systematically commenced to diagnose potentially undiagnosed cases of hypertension or T2DM. In addition, shunted patients were routinely reviewed only at 2–3 months after surgery, and no validated objective outcome measures were used. Lack of age- and gender-matched population-based controls is also a limitation to our study.

More clinical studies are needed to explore the relationship between vascular disease and iNPH.
with systematic evaluation of blood pressure and blood glucose levels. Does aggressive treatment of hypertension or T2DM affect the symptoms or progression of iNPH? Are there potential genetic or environmental factors that would explain the high vascular comorbidity in iNPH?

To conclude, we present the incidence of iNPH in a defined population in Middle and Eastern Finland. We confirm a high comorbidity of arterial hypertension and T2DM in patients with iNPH. Although iNPH was associated with a decreased risk of death in the patient cohort, a high frequency of T2DM caused excess mortality in these patients. Additionally, dementia was rather common as a cause of death in patients with iNPH.
5 APOE4 predicts amyloid-β in cortical brain biopsy but not idiopathic normal pressure hydrocephalus

5.1 ABSTRACT

5.1.1 Objective
To investigate the association of apolipoprotein E (APOE) genotype, especially the APOE4 allele, (1) to idiopathic normal pressure hydrocephalus (iNPH), and (2) to amyloid-β (Aβ) plaques in cortical brain biopsies of presumed NPH patients with and without a final clinical diagnosis of Alzheimer’s disease (AD).

5.1.2 Methods
Altogether 202 patients with presumed NPH were evaluated by intraventricular pressure monitoring and frontal cortical biopsy immunostained against Aβ (134 semiquantified by Aβ plaques/mm²). The 202 patients and 687 cognitively healthy individuals were genotyped for APOE. The final clinical diagnoses in a median follow up of 3.9 years were: 113 iNPH (94 shunt-responsive, 16 shunt-non-responsive, 3 not shunted); 36 AD (12 mixed iNPH + AD); 53 others.

5.1.3 Results
The APOE genotypes distributed similarly in the 94 shunt-responsive and 16 non-responsive iNPH patients and healthy controls. In the multivariate analysis, the APOE4 allele correlated independently with Aβ plaques in the cortical biopsies (odds ratio 8.7, 95% confidence interval 3.6–20, p < 0.001). The APOE4 allele in presumed NPH predicted later AD as follows: sensitivity 61%; specificity 77%; positive predictive value 37%; negative predictive value 90%.

5.1.4 Conclusions
In presumed NPH patients, APOE4 associates independently with the presence of Aβ plaques in the frontal cortical biopsy. APOE4 is not a risk factor for iNPH and does not predict the response to shunt. Our data further supports the view that the iNPH syndrome is a distinct dementing disease.

5.2 INTRODUCTION

Normal pressure hydrocephalus (NPH) is an uncommon dementing disorder in the elderly, presenting with enlarged ventricles but normal or slightly elevated CSF pressure. NPH is characterised by three clinical symptoms: gait disorder, impaired cognition and urinary incontinence (2, 3). Symptoms are often relieved, or even reversed, by CSF shunt insertion, the treatment of choice at present (226). Idiopathic NPH (iNPH) is a form of NPH without any known predisposing factors, such as brain injury, subarachnoid haemorrhage, or meningitis (7). Various procedures to evaluate CSF dynamics in patients with presumed NPH are used to identify those who would benefit from CSF shunting. These include the CSF tap test, external lumbar drainage test (119), infusion tests (119, 120), and intraventricular (87) or intracranial (70) pressure (ICP) monitoring. The most frequent differential diagnoses of iNPH are Alzheimer’s disease (AD) and vascular dementia (87).

In NPH patients, ICP monitoring and shunt insertion, both requiring a burr hole, allow for a small brain biopsy to assess the histological signs of AD and other dementias (87). AD is characterised by beta-amyloid (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau (HPτ) in the brain of patients with amnesic cognitive decline (146, 147, 227). The amyloid cascade hypothesis suggests that Aβ starts to accumulate even decades before the clinical manifesta-
tions of AD (148, 228). During life, Aβ can be detected with brain biopsy (87, 153) and evaluated by positron emission tomography (PET) utilising the 11C-labelled Pittsburgh compound B (150).

Apolipoprotein E (apoE) has a significant role in neurobiology (105). There are three different apoE isoproteins coded by three different APOE alleles (ε2, ε3, and ε4) with six different genotypes (101). The APOE genotypes correlate with Aβ accumulation in amyloid-targeted PET (229, 230) and in autopsy studies (231–235). To date, the APOE4 allele is the most important independent genetic risk factor for late onset AD (102, 106, 107, 236, 237) (http://www.alzgene.org). APOE4 is also associated with other neurological disorders (105, 238) including iNPH (22, 23). To date, the possible association of APOE and iNPH has been explored in only two small cohorts.

The objectives of this study were to determine:
1. the distribution of APOE genotypes in 94 shunt responsive iNPH patients and in 687 cognitively healthy controls,
2. the predictive value of APOE genotypes in the shunt response of 110 shunted iNPH patients,
3. the predictive value of APOE4 allele for the presence of Aβ plaques in frontal cortical biopsies of 199 patients with presumed NPH and for the final clinical diagnosis of AD.

5.3 METHODS

5.3.1 Catchment population of the Kuopio University Hospital
Neurosurgery of Kuopio University Hospital (KUH) is the sole provider of full time acute and elective neurosurgical services for the KUH catchment population in Middle and Eastern Finland (239). The KUH area contains four central hospitals with neurological units and catchment areas of their own. During the recruitment period of the present study, from 1993 to 2009, the geographic area remained the same, the population decreased from 874,228 to 842,496, and the proportion of men remained unchanged at 49%.

5.3.2 Kuopio NPH Registry
Patients fulfilling the following criteria were further assessed in KUH Neurosurgery as presumed NPH patients: (1) primary evaluation and examination by a neurologist; (2) one to three symptoms suggestive of NPH (gait disorder, cognitive impairment, and urinary incontinence); and (3) NPH-related brain imaging findings. Since 1993, the diagnostic workup of KUH Neurosurgery for presumed NPH has included a clinical examination, computed tomography (CT) or magnetic resonance imaging (MRI) scan and 24h intraventricular ICP monitoring together with a right frontal cortical biopsy. Kuopio NPH Registry (http://www.uef.fi/nph) consists of all presumed NPH patients from the KUH catchment population since 1993 with (a) clinical baseline and follow-up data and (b) other hospital diagnoses, medications, causes of death from national registries, and neuropathological findings.

5.3.3 Participants
All patients from 1993 to 2009 with presumed NPH, a frontal cortical brain biopsy, and a venous blood sample available until November 2009 (n = 202) from the Kuopio NPH Registry were included in this study (Table 6, Figure 11). The control population consisted of 687 individuals from Eastern Finland with no signs of cognitive decline on interview or neuropsychological testing (240, 241) (Table 6).
Table 6. Clinical characteristics, amyloid-β (Aβ) and hyperphosphorylated tau (HPt) proteins in right frontal cortical brain biopsy, and apolipoprotein E (APOE) genotypes of 202 presumed normal pressure hydrocephalus (NPH) patients. APOE genotypes of 687 cognitively healthy controls also presented.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Presumed NPH and cortical biopsy sample available (n = 202)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Final clinical diagnosis of iNPH (n = 113)</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Shunt responder</td>
<td>Shunt nonresponder</td>
</tr>
<tr>
<td>n</td>
<td>94</td>
<td>16</td>
</tr>
<tr>
<td>Median age, yr (range, yr)</td>
<td>70 (43–83)</td>
<td>71 (64–84)</td>
</tr>
<tr>
<td>Females, %</td>
<td>56</td>
<td>81</td>
</tr>
<tr>
<td>Median follow-up time, yr (range, yr)</td>
<td>3.3 (0.8–17)</td>
<td>5.1 (1.2–17)</td>
</tr>
<tr>
<td>Leading symptom, n (%)</td>
<td>Gait disorder</td>
<td>Memory impairment</td>
</tr>
<tr>
<td>Aβ – HPt –</td>
<td>63 (67)</td>
<td>9 (56)</td>
</tr>
<tr>
<td>Aβ + HPt –</td>
<td>13 (14)</td>
<td>3 (19)</td>
</tr>
<tr>
<td>Aβ + HPt +</td>
<td>16 (17)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Aβ – HPt +</td>
<td>2 (2)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Aβ – HPt +</td>
<td>53 (56)</td>
<td>10 (63)</td>
</tr>
<tr>
<td>Aβ + HPt –</td>
<td>25 (27)</td>
<td>5 (31)</td>
</tr>
<tr>
<td>Aβ + HPt +</td>
<td>9 (10)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Aβ – HPt +</td>
<td>7 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Immunoreactivity, n (%)</td>
<td>Aβ – HPt –</td>
<td>Aβ + HPt –</td>
</tr>
<tr>
<td>e2/e3</td>
<td>6 (6)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>e2/e4</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>e3/e3</td>
<td>70 (75)</td>
<td>11 (69)</td>
</tr>
<tr>
<td>e3/e4</td>
<td>17 (18)</td>
<td>3 (19)</td>
</tr>
<tr>
<td>e4/e4</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

\(^{a}\)112 patients did not develop AD in the follow-up and 1 patient had insufficient patient data available to diagnose or exclude AD

\(^{b}\)1 patient deceased before shunt operation, 1 refused, and 1 was not shunted due to heavy comorbidities

\(^{c}\)Patients with a clinical diagnosis of iNPH (11 shunted, 7 benefited), who developed AD in the follow-up

\(^{d}\)Of the 199 presumed NPH patients with sufficient follow-up data for a final clinical diagnosis of AD

\(^{e}\)Cases with other neurodegenerative diagnosis than iNPH or AD, including 2 cases with insufficient data to diagnose or exclude AD

\(^{f}\)9 cases did not have sufficient patient data to determine a leading symptom.

iNPH = idiopathic NPH, AD = Alzheimer's disease, APOE = apolipoprotein E
5.3.4 Shunt treatment and shunt response
A ventriculoperitoneal shunt (with Codman–Hakim medium pressure valve or PS Medical medium pressure valve, after revision in a few cases to low or high pressure valve) was inserted in 137 of 202 presumed NPH patients. Indications for the shunt treatment were: (1) basal ICP >10 mmHg but <20 mmHg continuously or (2) the presence of any A waves or more than 30% B waves during the 24h ICP monitoring period when basal pressure was between 5 and 10 mmHg (207).

The clinical response to shunt was evaluated at 2–3 months at the KUH Neurosurgery outpatient clinic. Thereafter, patients were re-examined only if they developed symptoms suggestive of shunt malfunction or progression of disease. Any subjective or objective improvement in patient gait, memory or urinary continence was graded as a positive shunt response (87).

5.3.5 Final clinical diagnosis
Patients were followed-up until death or the end of November 2010, for a median follow-up time of 3.9 years (range 0.2–17.3 years). For the final clinical diagnosis, all available clinical data from the hospitals in the KUH catchment area were retrospectively reviewed by a neurologist. Possible
or probable AD was diagnosed in 24 patients according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS–ADRDA) and Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (87, 242, 243). iNPH was diagnosed in 113 patients according to the following criteria: abnormal ICP findings indicating shunt surgery; no factors causing secondary NPH; and no clinical AD at the end of follow-up. In addition, there were 12 mixed cases of iNPH and AD (Table 6).

5.3.6 Immunohistochemical staining and histological evaluation of brain biopsy
The 202 paraffin embedded biopsy samples were sectioned and stained with haematoxylin–eosin and immunostained with monoclonal antibodies directed at Aβ (6F/3D) and HPτ (AT8), as described previously (87). In the histological evaluation, immunoreactivity for Aβ and HPτ in all samples was graded as present or absent by a neuropathologist. In 134 samples, Aβ was semiquantified by counting fleecy, diffuse and dense plaques in the whole cortical grey matter seen in the biopsy under light microscopy (magnification X 100) and dividing the total number of Aβ plaques by the area of grey matter (mm²).

5.3.7 APOE genotyping
Genomic DNA was extracted from 200µl of venous blood (Illustra Blood GenomicPrep Mini Spin Kit; GE Healthcare, Little Chalfont, UK) and stored at −20°C. A PCR method was used in the analysis, as described previously (244). APOE genotyping was carried out using the ABI3100 analyser (Applied Biosystems, Carlsbad, California, USA) and allele peaks were determined using GeneMapper software (Applied Biosystems).

5.3.8 Statistical analysis
Differences between groups were analysed with the χ² test for nominal variables and with the Mann–Whitney U test for continuous variables. Univariate and multivariate logistic regression analysis was used to evaluate the association of APOE4 status and other covariates with the three end variables: final diagnosis of iNPH in presumed NPH patients; presence of amyloid in cortical biopsy; and final diagnosis of AD in presumed NPH patients.

Patients with both iNPH and AD as the final clinical diagnoses were excluded from the analysis of APOE in shunt responsive iNPH patients and cognitively healthy controls, and from the analysis of shunt response, but were included in all other analyses. PASW Statistics for Mac (V.18.0.3, SPSS Inc.) was used and the level of significance was set at p < 0.05.

5.3.9 Ethical issues
The study was accepted by KUH Research Ethical Committee, The Finnish National Supervisory Authority for Welfare and Health, and The Finnish Ministry of Social Affairs and Health. All subjects or their proxies gave a written, informed consent prior to participation in the study.

5.4 RESULTS

5.4.1 APOE and shunt responsive iNPH
The distribution of the APOE genotypes was similar in the 94 shunt responsive iNPH patients (12 developed AD during follow-up and were excluded) and in the 887 healthy elderly controls (p = 0.27) (Table 6). The proportion of APOE4 carriers was lower in the shunt responsive iNPH patients (19%) compared with the control population (28%) (p = 0.06). Both groups had a similar gender distribution (56% vs. 60% females) and median age (70 years vs. 70 years). There were fewer APOE4 carriers in patients with a final clinical diagnosis of iNPH (24%) compared with presumed NPH patients with a different final clinical diagnosis (44%; p = 0.008) (Table 6).

5.4.2 APOE and shunt nonresponsive iNPH
There were 16 iNPH patients who did not respond to shunt. They did not develop clinical AD in a median follow-up time of 5.1 years (Table 6). The 94 shunt responsive and 16 nonresponsive iNPH patients had a similar distribution of APOE genotypes (p = 0.47) and proportion of APOE4 carriers
(19% vs. 19%; \( p = 0.72 \)).

5.4.3 APOE and Aβ plaques in brain biopsy
There were 199 patients with APOE status, immunohistochemical evaluation of the right frontal cortical biopsy and sufficient follow-up data available to diagnose or exclude AD (Figure 11). Aβ plaques were detected in 88 cases (44%) at a median age of 75 years and no Aβ plaques were seen in 111 cases at a median age of 71 years. APOE4 allele was present in 60 cases (30%) (Figure 11). In the multivariate logistic regression analysis (Table 7), independent predictors of Aβ plaques were APOE4 (odds ratio (OR) 8.7, 95% confidence interval (CI) 3.8–20), age at biopsy (OR/year 1.08, 95% CI 1.03–1.1) and final clinical diagnosis of AD (OR 7.9, 95% CI 2.3–27). Aβ load (plaques/mm²), semiquantified in 134 cases, was higher \( (p < 0.001) \) in APOE4 carriers (mean 12) than in non-APOE4 carriers (mean 2.5).

5.4.4 APOE and final clinical diagnosis of AD
Of the 199 patients, 24 had a final clinical diagnosis of AD at a median follow-up time of 3.3 years and, in addition, 12 iNPH patients developed AD at a median follow-up time of 2.9 years. Of the combined 36 AD cases, 22 (61%) were APOE4 carriers. In the multivariate logistic regression analysis (Table 7), memory impairment at biopsy (OR 9.3, 95% CI 3.4–26), clinical diagnosis of iNPH (OR 0.19, 95% CI 0.06–0.55), both Aβ plaques and HPt (OR 9.2, 95% CI 2.0–43), or Aβ plaques without HPt (OR 5.8, 95% CI 1.6–22) in the cortical brain biopsy were independent predictors of AD. In univariate logistic regression, APOE4 associated with AD (OR 4.3, 95% CI 2.0–9.3, \( p < 0.001 \)), but when adjusted for other covariates in a multivariate model, the OR halved (OR 1.8, 95% CI 0.61–5.5, \( p = 0.23 \)), which was mainly explained by inclusion of cortical pathology to the analysis (Table 7). The APOE4 allele in presumed NPH predicts AD as follows: sensitivity 61%; specificity 77%; positive predictive value 37%; negative predictive value 90%.
Table 7: Multivariate logistic regression analyses of 202 presumed normal pressure hydrocephalus (NPH) patients with a frontal cortical biopsy and apolipoprotein E (APOE) genotype available.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>iNPH as end variable (n = 170)</th>
<th>Aβ as end variable (n = 199)</th>
<th>AD as end variable (n = 192)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Odds ratio (95% CI)</td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>Age at biopsy/year</td>
<td>1.02 (0.98–1.07)</td>
<td>0.35</td>
<td>1.08 (1.03–1.13)</td>
</tr>
<tr>
<td>Male</td>
<td>79</td>
<td>1</td>
<td>Ref</td>
</tr>
<tr>
<td>Female</td>
<td>91</td>
<td>2.13 (0.99–4.61)</td>
<td>0.05</td>
</tr>
<tr>
<td>APOE4 noncarrier</td>
<td>116</td>
<td>1</td>
<td>Ref</td>
</tr>
<tr>
<td>APOE4 carrier</td>
<td>54</td>
<td>0.73 (0.29–1.82)</td>
<td>0.50</td>
</tr>
<tr>
<td>Male</td>
<td>79</td>
<td>1</td>
<td>Ref</td>
</tr>
<tr>
<td>Female</td>
<td>91</td>
<td>2.13 (0.99–4.61)</td>
<td>0.05</td>
</tr>
<tr>
<td>APOE4 noncarrier</td>
<td>116</td>
<td>1</td>
<td>Ref</td>
</tr>
<tr>
<td>APOE4 carrier</td>
<td>54</td>
<td>0.73 (0.29–1.82)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Leading symptom
- Other than gait disorder
- Memory impairment
  - Gait disorder: 86, 1, Ref
  - Memory impairment: 84, 4.41 (1.93–10.09), <0.001

Immunohistochemistry

| Aβ – HPt –                      | 89                            | 1                           | Ref                          | 53                  | 1                        | Ref |
| Aβ + HPt –                      | 57                            | 1.01 (0.36–2.80)            | 0.99                         | 105                 | 5.84 (1.56–21.83)         | 0.009 |
| Aβ + HPt +                      | 23                            | 0.61 (0.16–2.30)            | 0.47                         | 63                  | 9.21 (1.99–42.73)         | 0.005 |
| Aβ – HPt +                      | Excluded                      | 50                           | 0.81 (0.31–2.11)             | 0.67                | 50                        | 9.33 (3.38–25.77)         | <0.0001 |

NPH
- Not NPH
- iNPH
- sNPH

Alzheimer’s disease
- Not present
- Present

---

Notes:
- 22 sNPH patients, 7 iNPH patients with Aβ – HPt +, and 3 patients with insufficient follow-up data for a final clinical diagnosis of AD were excluded from the analysis.
- 3 non-AD patients with Aβ – HPt +, and 3 patients with insufficient follow-up data for a final clinical diagnosis of AD were excluded from the analysis.
- iNPH = idiopathic NPH, Aβ = amyloid beta protein, AD = Alzheimer’s disease, CI = confidence interval, APOE4 = apolipoprotein E ε4 allele, HPt = hyperphosphorylated tau protein, sNPH = secondary NPH.
DISCUSSION

This is the first sizeable cohort to assess the prognostic value of APOE alleles in iNPH patients. The APOE genotypes were equally distributed in shunt responsive iNPH patients and cognitively unimpaired controls, and did not predict the shunt response in iNPH patients. On the other hand, the APOE4 allele independently predicted Aβ plaques in the frontal cortical biopsy. The strengths of our study included: a large NPH cohort evaluated by 24h intraventricular ICP monitoring and frontal cortical biopsy; long term follow-up for possible development of AD or other forms of dementia; and a large cognitively unimpaired control population. Weaknesses included: a retrospective study; only six APOE4 homozygotes; a limited number of patients with no shunt response; systematic assessment of shunt response only at 2–3 months; dichotomised shunt response that overlooked the symptom triad of iNPH; and lack of validated objective outcome measures. It is clear that at least in prospective clinical trials, shunted patients should be followed-up routinely for a significantly longer time and with symptom specific outcome scales.

In contrast with our study, one small series suggested that APOE4 would be overrepresented in NPH patients (22). The study included 13 patients (mean age 72 years) with MRI and neuropsychological data suggestive of NPH and 108 apparently normal controls (mean age 81 years) with no neurological disorders. In our study, iNPH patients were evaluated by brain imaging, ICP monitoring and frontal cortical biopsy, and they were followed-up for a shunt response and for possible later clinical development of other forms of dementia. Thus with a notably larger iNPH patient cohort, more sound methodology and better differentiation of AD cases, our data is more reliable.

In the diagnostic workup of presumed NPH, APOE genotyping would be beneficial as APOE4 allele carriership indicates an increased risk of AD – or iNPH with concomitant AD. Importantly, the APOE4 allele does not exclude iNPH. Furthermore, APOE genotypes did not predict shunt response in our 121 shunted iNPH patients. Instead, in a series of 15 iNPH patients evaluated by gait analysis before and after CSF drainage, the APOE ε3/ε3 genotype seemed to predict improved gait (23). It should be noted that the cited study bears significant weaknesses, including a very small number of patients and no detection of primary or comorbid AD patients by means of brain biopsy, potentially invalidating the shunt response. Taking these facts into account, we regard our findings as more solid. However, the value of APOE genotyping in the prediction of shunt response should be evaluated in further studies with a longer follow-up of shunt response and by detailed analysis of gait, cognition (e.g., CERAD (245)) and urinary incontinence (e.g., structured NPH-questionnaires).

There is no consensus on the indications for shunt treatment in iNPH based on ICP diagnostics in literature. Some of our patients whose ICP monitoring did not support NPH might have benefited from a shunt. In contrast, a significant number of patients did not respond to shunt despite positive ICP findings. On the other hand, classification of true or definite iNPH often includes a positive response to shunt treatment. However, the response may be impeded by concomitant diseases or progression of iNPH beyond the possible point of no return. As the presumed NPH patients in our cohort were referred to KUH Neurosurgery presenting with NPH-related symptoms and history, in addition to CT and/or MRI scans with signs of enlarged brain ventricles disproportionate to the size of the sulci in the cerebral convexities, presumed NPH corresponds to possible iNPH according to the iNPH guidelines (7). Probable iNPH in the guidelines (7) translates to having a final clinical diagnosis of iNPH in our cohort as these patients had ICP abnormalities in further physiological diagnostic tests.

In our study, the APOE4 allele was strongly associated (OR 8.7) with Aβ plaques in the frontal cortical brain biopsy. Semiquantification of the Aβ load in the biopsies further emphasised the association, which is in line with previous autopsy studies (231–235) and recent PET imaging studies of Aβ (229, 230). Along with previous studies, we also found a significant association of age to Aβ deposits in the brain. Our study is the first to evaluate the association of APOE4 with Aβ plaques in the living human brain. Importantly, in our study and in a PET study for amyloid (230), accumulation of Aβ was detected approximately three years earlier in APOE4 carriers than in non-carriers. Aβ accumulation in the brain is a potential preclinical indicator for the risk of future AD, existing far earlier than any clinical signs of the disease (149, 246). Brain biopsy provides a unique research window to the living human brain and also allows the study of the relationship of APOE4 or
other biomarkers to molecular biology and the progress of AD. The current AlzGene meta-analysis (247) gives an OR of 3.68 for APOE4 versus APOE3 as a single biomarker in the prediction of AD. In our study, the OR was 4.3 in the univariate analysis, but addition of Aβ and HPτ pathology in the frontal cortical biopsy to the analysis, diluted the OR of APOE4 to 1.8. In a previous study of 198 patients with amnesic mild cognitive impairment and 98 AD patients, APOE4 dose correlated stronger with low CSF Aβ1–42 levels than with cognitive status (248). In the present study, there were six APOE4 homozygotes and three had a final clinical diagnosis of AD. The large variation in follow-up time of patients may have had an effect on the results concerning AD patients, as the principal risk factor for AD is age, and many more patients would have deteriorated if followed-up for longer. AD cannot be diagnosed solely on APOE genotype. However, APOE polymorphisms can predict Aβ pathology in the brain, and combined with other biomarkers, probably increase the diagnostic accuracy of AD.

Further research is needed to elucidate the genetics and pathobiology of iNPH as well as the potential diagnostic and prognostic biomarkers. In addition, prospective follow-up studies are required to clarify the predictive value of AD-related pathology and genetics to the long term response to CSF shunt treatment in iNPH.

In conclusion, in presumed NPH patients, APOE4 associates with the Aβ plaques in the frontal cortical biopsy. APOE4 is not a risk factor for iNPH and does not predict the response to shunt.
6 Cerebrospinal fluid biomarker and brain biopsy findings in idiopathic normal pressure hydrocephalus

6.1 ABSTRACT

6.1.1 Objective
To investigate the role of soluble amyloid precursor protein (sAPP) and amyloid beta (Aβ) isoforms, proinflammatory cytokines, and biomarkers of neuronal damage in the cerebrospinal fluid (CSF) in relation to brain biopsy Aβ and hyperphosphorylated tau (HPτ) findings in patients with idiopathic normal pressure hydrocephalus (iNPH) and Alzheimer’s disease (AD).

6.1.2 Methods
The study population comprised 102 patients with possible NPH with cortical brain biopsies, ventricular and lumbar CSF samples, and DNA available. The final clinical diagnoses were: 53 iNPH (91% shunt-responders), 26 AD (10 mixed iNPH-AD), and 23 others. Biopsy samples were immunostained against Aβ and HPτ. CSF levels of AD-related biomarkers (Aβ42, p-tau, total tau), non-AD-related Aβ isoforms (Aβ38, Aβ40), sAPP isoforms (sAPPα, sAPPβ), proinflammatory cytokines (several interleukins (IL), interferon-gamma, monocyte chemoattractant protein-1, tumor necrosis factor-alpha) and biomarkers of neuronal damage (neurofilament light and myelin basic protein) were measured. All patients were genotyped for APOE.

6.1.3 Results
Lumbar CSF levels of sAPPα were lower (p<0.05) in patients with shunt-responsive iNPH compared to non-iNPH patients. sAPPβ showed a similar trend (p=0.06). CSF sAPP isoform levels showed no association to Aβ or HPτ. CSF levels of AD-related biomarkers (Aβ42, p-tau, total tau), non-AD-related Aβ isoforms (Aβ38, Aβ40), sAPP isoforms (sAPPα, sAPPβ), proinflammatory cytokines (several interleukins (IL), interferon-gamma, monocyte chemoattractant protein-1, tumor necrosis factor-alpha) and biomarkers of neuronal damage did not associate to the brain biopsy findings, diagnosis, or shunt response. Higher lumbar/ventricular CSF IL-8 ratios (p<0.001) were seen in lumbar samples collected after ventriculostomy compared to the samples collected before the procedure.

6.1.4 Conclusions
The role of sAPP isoforms in iNPH seems to be independent from the amyloid cascade. No neuroinflammatory background was observed in iNPH or AD.

6.2 INTRODUCTION
Idiopathic normal pressure hydrocephalus (iNPH) is a progressive neurodegenerative disorder of unknown etiology in the elderly presenting with gait disorder, cognitive impairment, and urinary incontinence, with enlarged ventricles of the brain but normal or slightly elevated cerebrospinal fluid (CSF) pressure (2, 3). Currently there is no pathological hallmark for iNPH (144). Studies suggesting some potential genetic background of iNPH have been published (18, 20). The present treatment of choice in iNPH is CSF diversion with an implanted shunt that relieves or even reverses the symptoms. Various procedures to evaluate CSF dynamics in patients with possible iNPH are used to identify those who could benefit from CSF shunting. These include the CSF tap test, external lumbar drainage test, infusion tests, and intraventricular or intracranial pressure (ICP) monitoring.
(7, 70, 87, 120). The most frequent differential diagnoses of iNPH are atypical Alzheimer’s disease (AD) and vascular dementia (87, 88).

AD is characterized by the hallmark lesions of amyloid-β (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau (HPτ) in the brain of patients with amnestic cognitive decline (146, 147, 227). The amyloid cascade hypothesis states that Aβ starts to accumulate decades before the clinical manifestations of AD (148, 228).

In vivo, Aβ can be detected directly with brain biopsy (87, 153), or indirectly by observing low levels of Aβ in CSF (26). Fibrillar Aβ can also be evaluated by positron emission tomography (PET) utilizing the $^{11}$C-labelled Pittsburgh compound B (150) or $^{[18]}$F-flutemetamol (151).

Although common pathways for iNPH and AD have been proposed (83), the findings in genetic (249) and Aβ studies (87) suggest differences in etiologies of the two diseases. Aβ and HPτ in the CSF may help to differentiate iNPH and AD patient groups or detect comorbid AD in iNPH (26). In addition, these biomarkers have shown a potency to predict response to shunt in iNPH (174, 250).

Aβ originates from a cell membrane-spanning protein, amyloid precursor protein (APP), which has diverse roles in normal neuronal function (126). Soluble APP alpha (sAPPα) and beta (sAPPβ) result from the cleavage of APP by α- and β-secretases, respectively. Low CSF levels of sAPP isoforms have been reported in post-stroke and iNPH patients compared to AD and normal healthy controls (127–129, 251). In addition, sAPPα has shown a marked prognostic value for cognitive performance following shunt surgery (128), and subsequent increase of ventricular sAPP-levels has been noted in shunt-responders (129).

Abnormal levels of proinflammatory cytokines, such as interleukins (IL), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α), in CSF have been noted in various diseases of the nervous system, including AD (252). In iNPH, several proinflammatory cytokines have been studied, but none of them have proven to be useful in diagnostics (130). Lower levels of IL-1β in NPH compared to AD were reported in a single paper (131), while increased levels of IL-4 and IL-10 were reported in patients with NPH compared to healthy individuals in another study, but no significant difference was seen between NPH and other dementias (133). No differences were found between NPH and AD or healthy controls in studies comparing the levels of IL-8, IL-10, IL-12 (p40 and p70), IFN-γ, and transforming growth factor-β1 (TGF-β1) (129, 134). However, iNPH patients did show increased levels of MCP-1 compared to healthy individuals (129). Prior to treatment, higher TNF-α concentrations (and subsequent normalization after shunting) in CSF were observed in NPH patients compared to healthy controls in a single study (132); however, these results did not replicate in a more recent study with solely idiopathic NPH patients (135).

Elevated levels of neurofilament light protein (NFL) in the CSF, indicating neuronal death and axonal loss, have been found in NPH and secondary NPH in several studies (129, 137–140). Increased levels of myelin basic protein (MBP) in the CSF is a well-established biomarker for demyelination and myelin damage in the central nervous system (253). Furthermore, elevated levels of MBP have been reported in NPH (254).

To our knowledge, studies assessing the association between proinflammatory cytokines and biomarkers of neuronal damage in CSF, and cortical brain biopsy have not been published to date.

The objectives of the current study were:
1. to determine the levels of AD-related biomarkers (Aβ42, p-tau, total tau), non-AD-related Aβ isoforms (Aβ38, Aβ40), sAPP isoforms (sAPPα, sAPPβ), proinflammatory cytokines (IL-1β, 2, 4, 5, 8, 10, 12p70, and 13, IFN-γ, MCP-1, TNF-α) and biomarkers of neuronal damage (NFL, MBP) in lumbar and ventricular CSF, and how they correlate;
2. study the relationship between the CSF biomarkers and cortical brain biopsy;
3. assess the diagnostic and prognostic value of the CSF biomarkers in iNPH and AD.

### 6.3 METHODS

#### 6.3.1 Ethics statement

This study was approved by the Kuopio University Hospital (KUH) Research Ethical Committee, The Finnish National Supervisory Authority for Welfare and Health, and The Finnish Ministry of
Social Affairs and Health. All participants or their proxies gave a written, informed consent prior to participation in the study. If the clinician suspected dementia to significantly affect the capacity of the patient to consent, a next of kin, caretaker or guardian consented on behalf of the participant. When a consent was obtained from a participant’s proxy, the patient’s own opinion was inquired and considered, and no patient was recruited against their will.

6.3.2 Kuopio NPH Registry and protocol
Neurosurgery of KUH solely provides full-time acute and elective neurosurgical services for the KUH catchment population in Middle and Eastern Finland. In addition, the KUH area contains four central hospitals with neurological units and catchment areas of their own.

Patients fulfilling the following criteria were further assessed in KUH Neurosurgery as possible NPH patients: (1) primary evaluation and examination by a neurologist indicating NPH; (2) one to three symptoms suggestive of NPH (gait disorder, cognitive impairment, urinary incontinence); and (3) NPH related brain imaging findings (enlarged ventricles (Evans’ index > 0.3) together with obliterated cortical sulci). The diagnostic workup protocol of KUH Neurosurgery for possible NPH included a clinical examination, computed tomography (CT) or magnetic resonance imaging (MRI) scan, and 24h intraventricular ICP monitoring together with a frontal cortical brain biopsy. Kuopio NPH Registry (www.uef.fi/nph) consists of all evaluated possible NPH patients from the KUH catchment population since 1993.

The ICP criteria for shunt treatment in iNPH patients were (1) a basal ICP pressure between 10 and 20 mmHg continuously, or (2) the presence of A-waves or more than 30% B-waves during the 24h monitoring, when basal pressure was between 5 and 10 mmHg (207).

6.3.3 Participants
Altogether 102 patients, 51 men and 51 women, with a median age of 74.6 years (range 47–87 years) from the Kuopio NPH Registry with cortical brain biopsy, APOE genotype, and a ventricular CSF sample available were included in the study (Table 8). 63 patients were diagnosed with iNPH according to the protocol above, and were shunted with ventriculoperitoneal shunt (PS Medical medium pressure or adjustable valve). The clinical response to shunt was evaluated at 2–3 months after surgery, and any subjective or objective improvement in patient gait, memory or urinary continence was graded as a positive shunt response. Clinical AD was diagnosed according to a protocol described earlier (87, 88) in 26 patients (including 10 patients with initial/primary diagnosis of iNPH) in a median follow-up time of 2.3 years (range 0.2–6.2 years).
Table 8. Characteristics, brain biopsy findings, and apolipoprotein E ε4 (APOE-ε4) statuses of 102 patients with possible normal pressure hydrocephalus (NPH).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Final clinical diagnosis of iNPH (n = 53)</th>
<th>Possible NPH (n = 102)</th>
<th>No diagnosis of iNPH (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shunt responder</td>
<td>Shunt nonresponder</td>
<td>Mixed iNPH + AD</td>
</tr>
<tr>
<td>n</td>
<td>48</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Age (years) (median (range))</td>
<td>72.7 (63.8–87.3)</td>
<td>82.8 (79.9–86.2)</td>
<td>78.4 (69.8–86.7)</td>
</tr>
<tr>
<td>Women (n (%))</td>
<td>23 (48)</td>
<td>3 (60)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Follow-up time (years) (median (range))</td>
<td>2.51 (0.82–6.22)</td>
<td>2.16 (1.27–3.21)</td>
<td>1.95 (0.99–3.60)</td>
</tr>
<tr>
<td>Leading symptom, n (%)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gait disorder</td>
<td>29 (73)</td>
<td>3 (100)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Memory impairment</td>
<td>9 (23)</td>
<td>0 (0)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Immunoreactivity (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ – HPτ –</td>
<td>24 (50)</td>
<td>2 (40)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Aβ + HPτ –</td>
<td>17 (35)</td>
<td>2 (40)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Aβ + HPτ +</td>
<td>4 (8)</td>
<td>1 (20)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Aβ – HPτ +</td>
<td>3 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>APOE-ε4 carriers (n (%))</td>
<td>9 (19)</td>
<td>2 (40)</td>
<td>7 (70)</td>
</tr>
</tbody>
</table>

*Sufficient data for the determination of a leading symptom was unavailable in 10 iNPH patients, 3 mixed iNPH + AD patients, 2 AD patients and 4 patients with other final clinical diagnosis.

APOE = apolipoprotein E gene, CSF = cerebrospinal fluid, NPH = normal pressure hydrocephalus, iNPH = idiopathic NPH, AD = Alzheimer's disease, Aβ = amyloid beta protein, HPτ = hyperphosphorylated tau protein.
6.3.4 Immunohistochemistry and histological evaluation

Biopsy samples representing frontal cortex and subcortical white matter were stained with hematoxylin–eosin and immunostained with monoclonal antibodies directed to Aβ (6F/3D) and HPτ (AT8) as described earlier in detail (87). Positive Aβ immunostain was further quantified and reported as the ratio of area covered by Aβ to total area of cortex in the biopsy as described earlier (26).

6.3.5 APOE genotyping

DNA was extracted from venous blood using commercial kit according to manufacturer’s protocol (Illuma Blood GenomicPrep Mini Spin Kit, GE Healthcare, Little Chalfont, UK). A standard PCR method was used in the APOE genotyping (244).

6.3.6 CSF samples and biomarker analyses

The ventricular CSF samples were collected immediately after the placement of the intraventricular catheter (first 1 mL discarded) in the ICP measurement procedure. In addition, a lumbar CSF sample was available in 49 patients. The lumbar samples were obtained through a lumbar puncture prior to the ICP measurement protocol (12 patients) or 24–48 hours after introducing the ventricular catheter (37 patients).

Levels of AD biomarkers (Aβ42, p-tau, total tau) were measured from the CSF samples using commercial ELISA kits (Innotest β-amyloid1–42, Innotest Phosphtau(181P), Innotest Tau-Ag, Innogenetics, Ghent, Belgium) according to the manufacturer’s protocol at a validated laboratory in Neurology (www.uef.fi/neuro), University of Eastern Finland, Kuopio, Finland as described earlier (26).

Aβ isoforms (Aβ38, Aβ40), sAPP isoforms (sAPPα, sAPPβ), and the proinflammatory cytokines (IL 1β, 2, 4, 5, 8, 10, 12p70, and 13, IFN-γ, MCP-1, TNF-α) were analyzed utilizing commercially available multiplexed assays (Meso Scale Discovery, Gaithersburg, MD, USA) (129, 255), and NFL and MBP concentrations were measured using commercial ELISA kits (NF-Light, UmanDiagnostics, Umeå, Sweden, and ACTIVE MBP, Diagnostic Systems Laboratories, Webster, TX, USA, respectively). All analyses were performed according to the manufacturers’ protocols by board-certified laboratory technicians at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal. Each set of biomarker measurements was performed on one day, using one batch of reagents.

All clinical, immunohistochemical, and laboratory analyses were performed blinded to the result information of each other.

6.3.7 Statistical analysis

Nonparametric Kruskal–Wallis H and Mann–Whitney U tests were used for comparing CSF levels of measured biomarkers between different groups, and the Wilcoxon signed-rank test for comparisons of two related samples. To define the correlation between different proinflammatory cytokines, Pearson correlation with Bonferroni correction (k = 55) was applied.

Patients with tauopathy but no amyloid (n = 4) were excluded from the analyses of different biomarkers in relation to biopsy findings, and iNPH patients with comorbid AD (n = 10) and iNPH patients with negative shunt-response (n = 5) from the analyses comparing true iNPH patients to non-iNPH patients. Some cytokines were below the lower limit of detection of the assays and graded as zero concentration in statistical analyses. One patient had an insufficient CSF sample for the analysis of sAPPs, and another for the analysis of Aβ isoforms Aβ38 and Aβ40.

IBM SPSS Statistics for Mac (version 19.0.0.2, IBM, Armonk, NY, USA) was used in the statistical analyses. The level of significance was set at p < 0.05.

6.4 RESULTS

6.4.1 Aβ and sAPP isoforms

Lumbar CSF levels of sAPPα were significantly lower (p < 0.05) in patients with iNPH and a positive shunt response compared to non-iNPH patients, while sAPPβ showed a similar tendency (p = 0.06, Table 9, Figure 12B and 12D). However, no such association was seen for sAPP isoforms in

IBM SPSS Statistics for Mac (version 19.0.0.2, IBM, Armonk, NY, USA) was used in the statistical analyses. The level of significance was set at p < 0.05.
ventricular CSF samples ($p = 0.37–0.47$, Table 9, Figure 12A and 12C). In iNPH patients, a tendency towards lower levels of sAPPα and sAPPβ were observed ($p = 0.23–0.49$) in shunt-responders compared to nonresponders in ventricular and lumbar CSF (Table 9).

Ventricular CSF levels of Aβ42 differed ($p = 0.003$) between different brain biopsy groups (Table 10, Figure 13C). Patients with positive Aβ and HPτ immunoreactivity in the cortical brain biopsy showed significantly lower CSF levels of Aβ42 compared to the Aβ positive and HPτ negative group (post hoc $p = 0.008$), and to the Aβ and HPτ negative group (post hoc $p = 0.005$, Table 10, Figure 13C). Similar associations were seen in lumbar samples (data not shown). Quantified Aβ load showed a negative correlation with the levels of CSF Aβ42 in ventricular (Pearson’s $r = -0.295$, $p = 0.003$) and lumbar (Pearson’s $r = -0.356$, $p = 0.01$) samples (Figure 14). However, the CSF levels of other Aβ isoforms (Aβ38, Aβ40) and sAPP isoforms (sAPPα, sAPPβ) did not correlate ($p = 0.59–0.95$) with the presence of Aβ or HPτ in the biopsy (Table 10, Figure 13A–B). Furthermore, there was no correlation between the levels of sAPP isoforms in the CSF and Aβ load in cortical brain biopsy ($p = 0.84–0.92$). There were no statistically significant differences between the levels of CSF Aβ or tau biomarkers in shunt-responding and nonresponding iNPH patients.
Table 9. Cerebrospinal fluid (CSF) biomarker levels of 102 patients with possible normal pressure hydrocephalus (NPH).

<table>
<thead>
<tr>
<th>CSF Biomarker*</th>
<th>Final clinical diagnosis of iNPH (n = 53)</th>
<th>Possible NPH (n = 102)</th>
<th>No diagnosis of iNPH (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shunt responder</td>
<td>Shunt non-responder</td>
<td>Mixed iNPH + AD</td>
</tr>
<tr>
<td>Aβ42 Lumbar</td>
<td>587 (141)</td>
<td>563 (241)</td>
<td>487 (256)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>476 (203)</td>
<td>428 (250)</td>
<td>450 (134)</td>
</tr>
<tr>
<td>P-tau 181</td>
<td>35.3 (15.5)</td>
<td>38.0 (14.8)</td>
<td>41.1 (22.1)</td>
</tr>
<tr>
<td>Lumbar</td>
<td>77.1 (51.7)</td>
<td>50.4 (14.3)</td>
<td>93.1 (39.9)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>239 (156)</td>
<td>255 (121)</td>
<td>211 (135)</td>
</tr>
<tr>
<td>Total tau</td>
<td>1210 (1186)</td>
<td>562 (443)</td>
<td>1500 (1320)</td>
</tr>
<tr>
<td>sAPPα Lumbar</td>
<td>217 (156)</td>
<td>472 (560)</td>
<td>350 (137)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>237 (241)</td>
<td>374 (484)</td>
<td>261 (193)</td>
</tr>
<tr>
<td>sAPPβ Lumbar</td>
<td>84.3 (63.4)</td>
<td>193 (200)</td>
<td>102 (44.9)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>88.3 (92.3)</td>
<td>169 (235)</td>
<td>110 (92.6)</td>
</tr>
<tr>
<td>IL-8 Lumbar</td>
<td>1101 (2440)</td>
<td>318 (374)</td>
<td>85.7 (73.1)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>20.2 (20.8)</td>
<td>33.0 (51.3)</td>
<td>23.0 (5.76)</td>
</tr>
<tr>
<td>IL-8 ratio</td>
<td>132 (446)</td>
<td>55.0 (84.1)</td>
<td>3.05 (2.01)</td>
</tr>
<tr>
<td>MCP-1 Lumbar</td>
<td>3398 (2865)</td>
<td>1876 (766)</td>
<td>785 (515)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>748 (280)</td>
<td>1096 (688)</td>
<td>784 (187)</td>
</tr>
<tr>
<td>TNF-α Lumbar</td>
<td>4.38 (7.80)</td>
<td>1.88 (1.40)</td>
<td>0.50 (0.86)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>0.31 (0.62)</td>
<td>0.28 (0.63)</td>
<td>0.25 (0.55)</td>
</tr>
<tr>
<td>NFL Lumbar</td>
<td>2511 (1798)</td>
<td>6545 (6242)</td>
<td>2153 (830)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>886 (681)</td>
<td>6692 (9723)</td>
<td>1993 (1715)</td>
</tr>
<tr>
<td>MBP Lumbar</td>
<td>117 (170)</td>
<td>27.0 (9.93)</td>
<td>17.5 (–)</td>
</tr>
<tr>
<td>Ventricular</td>
<td>8.22 (12.1)</td>
<td>12.9 (19.2)</td>
<td>50.5 (73.5)</td>
</tr>
</tbody>
</table>

*Mean (SD) CSF concentrations in ng/L. CSF = cerebrospinal fluid, Aβ42 = amyloid beta 1-42, p-tau 181 = tau phosphorylated at threonine 181, sAPP = soluble amyloid precursor protein, IL-8 = interleukin 8, MCP-1 = monocyte chemoattractant protein-1, TNF-α = tumor necrosis factor-alpha, NFL = neurofilament light protein, MBP = myelin basic protein.
Table 10. Lumbar and ventricular cerebrospinal fluid (CSF) biomarkers in different brain biopsy findings.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Immunoreactivity</th>
<th>n</th>
<th>Lumbar</th>
<th>Ventricular</th>
<th>Lumbar</th>
<th>Ventricular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aβ– HPτ–</td>
<td>Aβ+ HPτ–</td>
<td>Aβ– HPτ+</td>
<td>Aβ+ HPτ+</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>23</td>
<td>17</td>
<td>1</td>
<td>8</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>48</td>
<td>33</td>
<td>4</td>
<td>17</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Aβ38*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>682 (370)</td>
<td>754 (365)</td>
<td>923 (–)</td>
<td>849 (352)</td>
<td>739 (360)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>583 (612)</td>
<td>533 (305)</td>
<td>564 (544)</td>
<td>615 (464)</td>
<td>572 (499)</td>
<td></td>
</tr>
<tr>
<td>Aβ40*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>6105 (3007)</td>
<td>6,170 (2157)</td>
<td>8143 (–)</td>
<td>6735 (1916)</td>
<td>6272 (2521)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>4544 (3162)</td>
<td>4,768 (2203)</td>
<td>4858 (3598)</td>
<td>5101 (3082)</td>
<td>4721 (2855)</td>
<td></td>
</tr>
<tr>
<td>Aβ42*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>641 (184)</td>
<td>596 (167)</td>
<td>758 (–)</td>
<td>393 (108)</td>
<td>588 (187)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>529 (240)</td>
<td>485 (188)</td>
<td>463 (327)</td>
<td>320 (162)</td>
<td>477 (225)</td>
<td></td>
</tr>
<tr>
<td>P-tau 181*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>37.8 (15.9)</td>
<td>37.0 (15.0)</td>
<td>45.6 (–)</td>
<td>48.9 (16.0)</td>
<td>39.5 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>81.3 (97.3)</td>
<td>73.6 (47.3)</td>
<td>83.5 (23.3)</td>
<td>95.8 (57.1)</td>
<td>81.3 (75.5)</td>
<td></td>
</tr>
<tr>
<td>Total tau*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>238 (139)</td>
<td>246 (164)</td>
<td>287 (–)</td>
<td>296 (127)</td>
<td>251 (143)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>1423 (2386)</td>
<td>1054 (1104)</td>
<td>1387 (415)</td>
<td>1652 (1970)</td>
<td>1340 (1924)</td>
<td></td>
</tr>
<tr>
<td>sAPPα*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>300 (287)</td>
<td>288 (211)</td>
<td>178 (–)</td>
<td>323 (261)</td>
<td>298 (251)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>266 (283)</td>
<td>264 (263)</td>
<td>179 (190)</td>
<td>249 (184)</td>
<td>259 (256)</td>
<td></td>
</tr>
<tr>
<td>sAPPβ*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>112 (106)</td>
<td>110 (77.0)</td>
<td>92 (–)</td>
<td>106 (75.8)</td>
<td>110 (89.5)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>104 (116)</td>
<td>105 (107)</td>
<td>69.5 (65.2)</td>
<td>95.3 (76.6)</td>
<td>102 (105)</td>
<td></td>
</tr>
<tr>
<td>IL-8*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>852 (2299)</td>
<td>435 (732)</td>
<td>197 (–)</td>
<td>1218 (1901)</td>
<td>754 (1792)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>43.5 (165)</td>
<td>22.8 (24.3)</td>
<td>16.9 (8.04)</td>
<td>15.6 (10.0)</td>
<td>31.1 (114)</td>
<td></td>
</tr>
<tr>
<td>IL-8 ratio**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar/ventricular</td>
<td>154 (525)</td>
<td>36.9 (51.3)</td>
<td>9.12 (–)</td>
<td>154 (133)</td>
<td>112 (370)</td>
<td></td>
</tr>
<tr>
<td>MCP-1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>2994 (2628)</td>
<td>2519 (2354)</td>
<td>3059 (–)</td>
<td>3637 (3323)</td>
<td>2935 (2602)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>767 (391)</td>
<td>820 (304)</td>
<td>704 (191)</td>
<td>771 (165)</td>
<td>782 (327)</td>
<td></td>
</tr>
<tr>
<td>TNF-α*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>2.77 (4.94)</td>
<td>2.55 (2.85)</td>
<td>1.27 (–)</td>
<td>6.88 (11.2)</td>
<td>3.34 (5.91)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>0.28 (0.74)</td>
<td>0.22 (0.59)</td>
<td>0.36 (0.72)</td>
<td>0.34 (0.67)</td>
<td>0.27 (0.67)</td>
<td></td>
</tr>
<tr>
<td>NFL*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>2216 (2044)</td>
<td>3135 (3073)</td>
<td>1180 (–)</td>
<td>2479 (1987)</td>
<td>2557 (2419)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>1127 (1385)</td>
<td>2082 (4153)</td>
<td>618 (448)</td>
<td>1213 (1307)</td>
<td>1430 (2615)</td>
<td></td>
</tr>
<tr>
<td>MBP*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar</td>
<td>71.5 (77.6)</td>
<td>82.2 (71.6)</td>
<td>48.9 (–)</td>
<td>160 (275)</td>
<td>89.9 (131)</td>
<td></td>
</tr>
<tr>
<td>Ventricular</td>
<td>12.7 (24.2)</td>
<td>17.1 (33.5)</td>
<td>30.5 (23.5)</td>
<td>9.65 (18.7)</td>
<td>14.4 (26.8)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean (SD) CSF concentrations in ng/L. **Mean (SD). Only cases with CSF sample obtained after 24 h ICP monitoring included (n = 37). Aβ = amyloid beta protein, HP = hyperphosphorylated tau protein, Aβ38/40/42 = amyloid beta 1-38/40/42, p-tau 181 = tau phosphorylated at threonine 181, sAPP = soluble amyloid precursor protein, IL-8 = interleukin 8, MCP-1 = monocyte chemoattractant protein-1, TNF-α = tumor necrosis factor-alpha, NFL = neurofilament light protein, MBP = myelin basic protein.
Figure 12. Scatterplots of soluble amyloid precursor protein alpha (sAPPα) and beta (sAPPβ) concentrations in ventricular (A and C) and lumbar (B and D) cerebrospinal fluid (CSF) in patients with no diagnosis of idiopathic normal pressure hydrocephalus (iNPH) and patients with true (shunt-responsive) iNPH are presented. Cases are colour-labelled according to their APOE-ε4 status: blue dots (●) indicate APOE-ε4 carriers and red dots (●) indicate APOE-ε4 noncarriers. *P*-values were determined using a Mann–Whitney U test.
Figure 13. Scatterplots of amyloid beta 1-38 (Aβ38) (A), Aβ40 (B), and Aβ42 (C) in groups of positive/negative Aβ and hyperphosphorylated tau (HPτ) immunoreactivity in brain biopsy are presented. Cases are colour-labelled according to their APOE-ε4 status: blue dots (●) indicate APOE-ε4 carriers and red dots (●) indicate APOE-ε4 noncarriers. P-values were determined using a Kruskal–Wallis H test and post-hoc Mann–Whitney U test with Bonferroni correction. Only statistically significant p-values are shown.
Figure 14. Scatterplots of ventricular (A) and lumbar (B) cerebrospinal fluid (CSF) amyloid beta 1-42 (Aβ42) levels in relation to the percentage of Aβ area in frontal cortical brain biopsies are presented. Cases are colour-labelled according to their APOE-ε4 status: blue dots (●) indicate APOE-ε4 carriers and red dots (●) indicate APOE-ε4 noncarriers. Correlation coefficients and p-values were determined using Pearson correlation.

6.4.2 Proinflammatory cytokines
Several cytokines were present at concentrations below the lower limit of detection of the assay. All tested proinflammatory cytokines showed positive correlations between each other in ventricular and lumbar CSF samples. All ventricular and lumbar CSF IL-8 samples and all but one ventricular CSF MCP-1 sample were above the lower limit of detection, and the two cytokines and TNF-α were chosen for further analyses from the proinflammatory cytokines measured (Table 9 and 10).

Lumbar CSF samples showed higher IL-8 levels compared to ventricular samples (p < 0.001, Table 9 and 10). Moreover, lumbar/ventricular CSF IL-8 ratios (p < 0.001) were significantly higher in samples collected after the ventriculostomy and ICP measurement compared to those collected before the procedure (Table 9 and 10, Figure 15).

The levels of tested proinflammatory cytokines in the CSF showed no association to the presence of Aβ or HPτ in brain biopsy, diagnosis of iNPH or AD, or shunt response in iNPH patients (Table 9).

6.4.3 Biomarkers of neuronal damage
There was no significant relation of CSF NFL or MBP levels to the brain biopsy findings (Table 10) or to the diagnosis of iNPH or AD (Table 9). In iNPH, a tendency (p = 0.05) towards lower ventricular CSF NFL values was seen in shunt-responders (Table 9).
DISCUSSION

This is the first study to explore Aβ and sAPP isoforms, proinflammatory cytokines, and biomarkers of neuronal damage in the CSF in conjunction with cortical brain biopsy. The major finding in the current study was the demonstration of the independent role of sAPPα in iNPH, which is not explained by cortical Aβ pathology.

In iNPH, decreased levels of sAPP isoforms in lumbar CSF have been reported in previous studies (127–129). As predicted, the level of lumbar CSF sAPPα was lower, whereas sAPPβ showed a similar trend in patients with shunt-responsive iNPH compared to non-iNPH patients in our patient cohort. Interestingly, in ventricular samples no association with iNPH was noted, which could be explained by the ventriculostomy procedure as an invasive sample collection method or by concentration of proteins in lumbar CSF. However, the reason for unaffected ventricular levels of sAPP isoforms is uncertain, as the ventricular CSF could be expected to reflect the periventricular metabolism better than lumbar CSF. The pathobiological role of sAPP isoforms in iNPH seems to

Figure 15. Scatterplot of lumbar/ventricular cerebrospinal fluid (CSF) interleukin 8 (IL-8) ratio in individual cases in relation to the time difference between lumbar and ventricular samples is presented. Cases are colour-labelled according to whether lumbar CSF sample was collected before (yellow dot (●)) or after (red dot (●)) the ventricular sample.

6.5 DISCUSSION

This is the first study to explore Aβ and sAPP isoforms, proinflammatory cytokines, and biomarkers of neuronal damage in the CSF in conjunction with cortical brain biopsy. The major finding in the current study was the demonstration of the independent role of sAPPα in iNPH, which is not explained by cortical Aβ pathology.

In iNPH, decreased levels of sAPP isoforms in lumbar CSF have been reported in previous studies (127–129). As predicted, the level of lumbar CSF sAPPα was lower, whereas sAPPβ showed a similar trend in patients with shunt-responsive iNPH compared to non-iNPH patients in our patient cohort. Interestingly, in ventricular samples no association with iNPH was noted, which could be explained by the ventriculostomy procedure as an invasive sample collection method or by concentration of proteins in lumbar CSF. However, the reason for unaffected ventricular levels of sAPP isoforms is uncertain, as the ventricular CSF could be expected to reflect the periventricular metabolism better than lumbar CSF. The pathobiological role of sAPP isoforms in iNPH seems to
be unconnected to the amyloidogenic pathway as there was no correlation between sAPP isoform levels in the CSF and Aβ load in the cortical brain biopsy. The reason for the lowering of sAPP isoform levels in untreated iNPH remains unclear. However, as the levels are restored upon successful shunt treatment it has been hypothesized that the lowering may reflect metabolic impairment in brain tissue affected by iNPH (129). In any case, our data suggests that the observed APP changes are independent of Aβ pathology or are so early in the cascade that they do not reflect current tissue pathology.

As expected, CSF levels of Aβ42 showed a negative association and correlation to Aβ load in the brain, as published earlier (26), and as suggested by amyloid-imaging studies (256). In contrast, no such association was seen between the CSF levels of Aβ38 or Aβ40 with positive Aβ or HPτ immunoreactivity in the cortical brain biopsy. Our findings support the non-amyloidogenic role of Aβ38 and Aβ40 in the living human brain.

As predicted, AD patients had lower Aβ42 and higher p-tau levels in the CSF compared to non-AD patients, although the differences did not reach statistical significance. One explanation for this is that non-AD patients show similar CSF Aβ42 and p-tau findings as biomarkers of comorbid AD tissue pathology without clinical dementia of Alzheimer’s type. As no pathological hallmark lesions have been identified in iNPH (144), the role of Aβ and tau in iNPH remains elusive. However, there are patients with mixed pathologies i.e., patients with AD-related pathology and later dementia but still initial objective response for shunt surgery (88). Interestingly, patients with iNPH-AD had lowest levels of CSF Aβ42, and highest frequency of APOE-ε4 carriers. Eighty percent (8/10) of these patients showed a favourable response to shunt treatment.

In contrast to two previous studies (174, 250), we found no prognostic potential in the levels of CSF Aβ42 or tau in shunted iNPH patients. The differences in the results may be explained by the different sample collection time (most the samples in the current study were collected after ventriculostomy). It should also be noted that cited studies included fewer patients.

In iNPH, reports of abnormal levels of proinflammatory cytokines (IL-1β, IL-4, IL-10, MCP-1, TNF-α) in the CSF have been published (129, 131–133), while contradictory findings in studies comparing proinflammatory cytokines (IL-8, IL-10, IL-12 (p40 and p70), IFN-γ, TNF-α, TGF-β1) have also been reported (129, 134, 135). In the current study, we attempted to measure a wide panel of different proinflammatory cytokines from ventricular and lumbar CSF, and positive correlations between cytokines were seen. However, we also noted that most of the cytokines are present in CSF at concentrations that are close to or below the lower limit of detection of the assay. In fact, these low concentrations, which are technically challenging to measure, may explain some of the varying results in the published literature. Here, we focused on the cytokines that could be robustly quantified in at least a subset of samples, i.e., IL-8. No association of proinflammatory cytokines in the CSF with the diagnosis of iNPH or AD or the presence of Aβ or HPτ in brain biopsy was seen. In patients with iNPH, proinflammatory cytokines did not show a prognostic value in shunt surgery. Consequently, our data suggests the role of neuroinflammation in iNPH and AD to be of little importance. Instead, an inflammatory response was seen in lumbar CSF samples collected after the ventriculostomy and ICP measurement. In addition, increased CSF tau and p-tau levels were observed in lumbar samples obtained after ventriculostomy as reported in earlier studies (26, 140). In consequence, levels of biomarkers in post-ventriculostomy lumbar CSF may not reflect the true values of the biomarkers in these patients.

Previous studies have reported increased levels of CSF NFL in NPH (129, 137–140). In our cohort, NFL showed higher lumbar CSF levels in patients with shunt-responsive iNPH compared to non-iNPH patients (Table 9), but not to a significant degree. Interestingly, iNPH patients with positive shunt response showed a tendency towards lower NFL levels in ventricular CSF compared to shunt-nonresponsive iNPH patients. As NFL reflects subcortical axonal damage, perhaps high NFL could represent more severe and less recovering injury in the hydrocephalic brain.

The strengths of the current study included: a large NPH cohort evaluated by cortical brain biopsy, utilization of ventricular and lumbar CSF samples in the analyses, a wide panel of tested CSF biomarkers, and evaluation of clinical outcome and other dementing disorders in the follow-up. The limitations of this study included: a limited number of patients with no shunt response, systematic assessment of shunt response only at 2–3 months, dichotomised shunt response scale, lack of validated objective outcome measures, and lumbar CSF sample available only in half of
the cases. It is obvious that at least in a prospective research setting, shunted patients should be followed-up for a significantly longer time and validated outcome measures should be utilized. Five patients with diagnostic findings suggesting iNPH did not respond to shunt, possibly due to comorbidities or misdiagnosis, and thus these patients were excluded from the comparisons of ('true') iNPH patients and non-iNPH patients, in addition to the iNPH patients who were diagnosed with comorbid AD in the follow-up.

More basic science and clinical studies evaluating the biology and potential role as diagnostic and prognostic biomarker of sAPPα and β are needed in the future.

To conclude, the role of sAPP isoforms in iNPH seems to be unconnected to the Aβ cascade pathway, but rather may be explained by a metabolic failure or ischemia in the brain. No elevations in the levels of proinflammatory cytokines in the CSF were observed in the different diagnostic groups. Consequently, neuroinflammation in iNPH and AD require further study. None of the tested CSF biomarkers showed a tendency to discriminate between iNPH and non-iNPH patients or shunt-responders and nonresponders in iNPH in a clinical setting.
7 General discussion

This doctoral thesis studied currently the largest consecutive iNPH cohort through a unique window of brain pathology with the implementation of cortical brain biopsy. Spanning over three decades, a major research opportunity to assess long-term morbidity and mortality in iNPH was identified. The most important findings of the thesis were: defining the incidence of iNPH in Middle and Eastern Finland; confirmation of a high vascular comorbidity and how it affects the mortality and the causes of death in iNPH; clarification of the genetical background of iNPH regarding the APOE genotypes; describing the independent role of APP in iNPH; and the evaluation of neuroinflammation in iNPH.

iNPH is an uncommon disease. Previous hospital-based studies performed in the Netherlands (10), Scandinavia (12, 14), and the United States of America (15) have presented incidence figures between 0.5–1.2 shunted iNPH patients per 100,000 inhabitants per year. In this thesis, a mean incidence of 1.8 iNPH patients / 100,000 / year was reported during 1993–2010 in Middle and Eastern Finland with a growing trend. It is not known whether these figures truly indicate a higher incidence of iNPH in Finland compared to other countries, or if the observed disparity is due to differences in the design of the study or variety in the detection and selection of the patients. However, an even higher incidence of iNPH has been reported in population-based studies (13, 48). In persons aged 70 or older, the age-specific incidence of iNPH was notably higher at 15 cases / 100,000 / year. The possibility of iNPH should be considered in the differential diagnostics of gait and cognitive symptoms in the elderly, as most iNPH patients show a positive response to shunt surgery.

The most common reported comorbidity in patients with iNPH is systemic arterial hypertension (8, 9, 176–180). In accordance with previous studies, a high frequency of hypertension (52%) was observed in iNPH patients. In addition, T2DM was also frequent in persons with iNPH (23%). High vascular comorbidity reflected the causes of death in patients with iNPH: the most common causes of death were heart disease and cerebrovascular disease. Interestingly, despite the heavy burden of vascular disease, overall mortality was lower and estimated survival higher in patients with iNPH compared to non-iNPH patients in the patient cohort. Differential diagnostic options of iNPH such as AD and VCI carry worse prognosis compared to shunted iNPH patients. In future studies and in clinical practice, blood pressure monitoring and blood glucose tests (a fasting glucose test, a glucose tolerance test, or HbA1c) should be utilized to identify previously undiagnosed cases of hypertension and T2DM in iNPH patients, as T2DM increases the risk of death in the affected patients. The observed high vascular comorbidity supports the vascular theories of pathogenesis of iNPH (2.4.2 Pathophysiological theories).

As there are several published studies of potential familial iNPH, a genetic predisposition to iNPH seems likely (16–18, 20). Identifying the genetic background of iNPH would be beneficial in the diagnostics of iNPH and would shed light on the pathogenesis of the disease. Previously, APOE ε4 allele, the most important genetic risk factor for AD, was suggested to be overrepresented in iNPH in a single study (22). Even though the difference was statistically significant ($p = 0.02$), the cited study shows major limitations: only a small number of NPH patients ($n = 13$), no differentiation between idiopathic and secondary NPH, incomplete representation of APOE genotypes, and remarkably lower frequency of APOE ε4 in the controls compared to other studies. However, with a superior number of patients and healthy controls and more rigorous differential diagnostics, no differences between iNPH and control subjects were observed in the APOE genotypes in this doctoral thesis. As expected, APOE ε4 associated with the presence of Aβ plaques in the brain biopsy and the diagnosis of AD. APOE does not seem to have a major role in the pathogenesis of iNPH, however, APOE genotyping is often used in randomized clinical trials associated to neurodegenerative diseases, and in the future it may be useful in planning individualized therapy for the patients (257).

The effect of the amyloid cascade was further investigated in this thesis. Importantly, lower CSF
levels of sAPP isoforms were observed in shunt-responsive iNPH patients compared to non-iNPH subjects. This finding is in line with earlier studies of APP in iNPH (127–129). Although lumbar CSF sAPPα levels were lower \((p < 0.05)\) in patients with shunt-responsive iNPH compared to non-iNPH patients, a significant overlap of concentrations were observed in both patient groups, which limits the clinical value of the finding. Importantly, the differences in sAPP levels in the lumbar or ventricular CSF were not reflective of cortical Aβ pathology, as demonstrated in this doctoral thesis. Instead, CSF levels of Aβ42 and HPτ show weak to moderate correlation with frontal cortical brain biopsy findings, as has been demonstrated in previous studies (26) and in this thesis. Brain biopsy is a useful tool in the differential diagnostics of iNPH at least in a research setting and for atypical cases, when specific diagnosis is needed but cannot be made with support of conventional noninvasive diagnostic tools, such as an MRI scan (87, 170).

Neuroinflammation in iNPH has been studied by measuring the levels of proinflammatory cytokines in the CSF (129, 131–135). The results have been variable and none of the tested cytokines have proven to be useful in the diagnosis of iNPH (130). In this thesis, a wide panel of proinflammatory cytokines were examined from the lumbar and ventricular CSF of iNPH patients to identify possible novel biomarkers in iNPH, but no diagnostic or prognostic potential was observed. In consequence, neuroinflammation does not seem to have a measurable effect on iNPH at least from a clinical perspective. As predicted, a marked inflammatory response was seen in lumbar CSF samples collected after the ventriculostomy and ICP measurement procedure. As the CSF shunt treatment is associated with potentially lethal complications such as subdural haematoma and intracranial infections, there is critical need for future studies to identify noninvasive biomarkers to diagnose and to predict the response for shunt surgery in iNPH.

There are limitations regarding the results presented in this thesis. Retrospective design of the studies meant that some clinical data was unavailable (e.g. systematic blood pressure measurements or blood glucose tests to find previously undiagnosed cases of hypertension or T2DM). For shunted iNPH patients there were no routine long-term follow-up visits, and no validated objective outcome measures were used to evaluate shunt response. There is an important selection bias as the patients in the cohort were referred to a tertiary university hospital, which may have limited the number of patients with only mild or early symptoms and patients at the other end of the spectrum with very poor general health due to other diseases and potentially old age. In addition, age- and gender-matched population-based controls were not utilized in all the studies.

To conclude, while the understanding of the special clinical problem of iNPH has been expanded, the true pathogenesis of the disease remains a mystery. The most promising lines of future research explored in this doctoral thesis are the high vascular comorbidity and the role of sAPP isoforms. As a genetic predisposition to iNPH seems likely, a great potential lies in the genomic study of iNPH in the future.
8 Conclusions

In conclusion:

1.) The diagnosis of iNPH is uncommon in the total population (1.8 / 100,000 / year). The incidence of iNPH increases with ageing and the age-specific incidence in the inhabitants aged 70 or older is 15 / 100,000 / year in Middle and East Finnish populations (i).

2.) Systemic arterial hypertension and T2DM are frequent comorbidities in patients with iNPH, and the latter causes excess mortality in the affected patients. Heart disease and cerebrovascular disease are the most common causes of death in iNPH (i).

3.) APOE ε4 is not a genetic risk factor for iNPH and does not predict the response to shunt. Instead, the presence of APOE ε4 allele predisposes to concomitant AD pathology (ii).

4.) The role of sAPP isoforms in iNPH seems to be independent from the cerebral Aβ pathology. No neuroinflammatory findings were observed in patients with iNPH (iii).

Roman numerals refer to original publications.
9 References


71. Bateman GA. The pathophysiology of idiopathic normal pressure hydrocephalus: cerebral


84. Silverberg GD. Normal pressure hydrocephalus (NPH): ischaemia, CSF stagnation or both. Brain 2004 May;127(Pt 5):947-948.


100. Zannis VI, Just PW, Breslow JL. Human apolipoprotein E isoprotein subclasses are genetically


187. Chen BH, Drucker MD, Louis KM, Richards DW. Progression of normal-tension glaucoma
after ventriculoperitoneal shunt to decrease cerebrospinal fluid pressure. J Glaucoma 2014 Oct 27.


202. Low EV, Avery AJ, Gupta V, Schedlbauer A, Grocott MP. Identifying the lowest effective dose of acetazolamide for the prophylaxis of acute mountain sickness: systematic review and


Hanson AJ, Craft S, Banks WA. The APOE genotype: modification of therapeutic responses in


Idiopathic normal pressure hydrocephalus is a slowly progressive syndrome in the elderly characterized by gait disorder, cognitive deterioration, and urinary incontinence. Compared to earlier studies, a higher annual incidence of the syndrome was noted in a Finnish population with an increasing trend. High vascular comorbidity and mortality was observed without a major neuroinflammatory component. Apolipoprotein E genotypes did not differentiate patients with idiopathic normal pressure hydrocephalus from healthy age- and gender-matched controls.