Idiopathic normal pressure hydrocephalus (iNPH) is a rare condition with typical symptoms. The accumulation of β-amyloid (Aβ) plaques typical for Alzheimer’s disease (AD) is seen in iNPH as well, yet the underlying mechanisms are unknown. This thesis deciphers the accumulation of Aβ in iNPH on the level of protein processing as well as by investigating underlying gene expression. The findings presented in this thesis provide new information considering the formation of Aβ plaques in iNPH and reveal several interesting differences in molecular pathology between iNPH and AD.
Deciphering potential factors affecting β-amyloid pathology in idiopathic normal pressure hydrocephalus
TIINA LAITERÄ

Deciphering potential factors affecting β-amyloid pathology in idiopathic normal pressure hydrocephalus

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ABSTRACT:

Idiopathic normal pressure hydrocephalus (iNPH) is a rare condition which manifests as a typical clinical symptom triad, normal or slightly elevated cerebrospinal fluid (CSF) pressure, and evidence of ventriculomegaly in brain imaging. Its typical symptoms are gait difficulties, impaired cognition and urinary incontinence. The severity and combination of the symptoms vary and patients with suspected iNPH are a very heterogeneous group. The underlying disease pathogenesis is poorly understood, although iNPH has been shown to share some similarities with the brain pathology present in Alzheimer's disease (AD). In AD, the accumulation of β-amyloid (Aβ) plaques is considered a central clinical hallmark and similar Aβ plaques are frequently encountered in iNPH. Furthermore, AD and iNPH often co-occur. Nevertheless, the mechanisms leading to the accumulation of Aβ and its significance for disease progression in iNPH are thus far unknown.

The aim of this study was to decipher the molecular pathology of iNPH with the main focus on the accumulation of Aβ. We measured γ- and β-secretase (BACE1) activities in brain biopsies collected from iNPH patients and compared them with activities measured on AD brain samples. The iNPH samples were subdivided into groups according to Aβ deposition and AD samples according to disease severity based on Braak staging. We also investigated the expression of specific genes coding for proteins known to be crucial in the different steps of the Aβ production, in iNPH samples and non-demented controls. Soluble products of amyloid precursor protein (APP) processing (sAPPα/β) and soluble transthyrethin (sTTR) were measured from the CSF sampled from iNPH patients. In addition, a polygenic risk score was constructed from single nucleotide polymorphisms (SNP) based on AD genome-wide association studies (GWAS) and compared to Aβ deposition in iNPH. The correlation with the apolipoprotein E allele ε4 (APOE4) burden was evaluated separately.

In iNPH samples, the γ-secretase activity increased with increasing Aβ deposition. BACE1 activity remained unchanged, while the opposite was seen in AD samples. The expression of APP and ADAM10, encoding one of the α-secretases, was increased and the expression of TTR decreased in iNPH in comparison to non-demented controls. The CSF levels of sAPPα/β or sTTR did not correlate with brain pathology or shunt prognosis in iNPH. The polygenic risk score analysis did not display any correlation with Aβ deposition except for APOE4.

In conclusion, these results reveal potential differences in Aβ accumulation between iNPH and AD. In iNPH, the levels of APP and the alterations in Aβ production might be more crucial for Aβ accumulation than in AD. TTR seems to be less important as a neuroprotective factor in iNPH than in AD, where the production of TTR is enhanced in conjunction with the accumulation of Aβ. In iNPH, APOE4 predicts Aβ accumulation as is the case in AD, but it is not predictive for the condition itself. These data point to at least a partly different role for these factors in the pathogenesis of these two diseases.
Medical Subject Headings: Hydrocephalus, Normal Pressure; Pathology, Molecular; Alleles; Alzheimer Disease; Amyloid Precursor Protein Secretases/metabolism; Amyloid beta-Protein Precursor/metabolism; Apolipoproteins E; Biopsy; Brain; Disease Progression; Gene Expression; Gene Expression Profiling; Plaque, Amyloid
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List of the original publications

This dissertation is based on the following original publications:


*The authors contributed equally.

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Abbreviations

ABCA7 ATP-binding cassette, subfamily A member 7
AD Alzheimer's disease
ADAM A disintegrin and metalloprotease
ADI Alzheimer's Disease International
AICD APP intracellular domain
APH-1 Anterior pharynx defective 1
ApoE Apolipoprotein E
APP Amyloid precursor protein
APRP Amyloid precursor related protein
Aβ β-amyloid
BACE1 β site APP -cleaving enzyme 1
BBB Blood-brain barrier
BIN1 Bridging Integrator 1
CASS4 Cas scaffolding protein family member 4
CD2AP CD2 associated protein
CD33 CD33 molecule (gene), myeloid cell surface antigen CD33 (protein)
CELF1 CUGBP, Elav-like family member 1
CLU Clusterin
CNS Central nervous system
CR1 Complement component (3b/4b) receptor 1
CSF Cerebrospinal fluid
CSF-OP CSF opening pressure
CT Computer tomography
DESH Disproportionately enlarged subarachnoid space hydrocephalus
DSM-IV Diagnostic and Statistical Manual of Mental Disorders
EOAD Early-onset Alzheimer's disease
FAD Familial Alzheimer's disease
FERMT2  Fermitin family member 2
FRMD4A  FERM domain containing 4A
GWAS  Genome-wide association study
HLA-DRB5  Major histocompatibility complex, class II, DR beta 5
(H)Pτ  (Hyper-)phosphorylated τ
ICP  Intracranial pressure
(i)NPH  (Idiopathic) normal pressure hydrocephalus
INPP5D  Inositol polyphosphate-5-phosphatase
ISHCSF  International Society for Hydrocephalus and Cerebrospinal Fluid Disorders
IWG  International Working Group
KPI  Kunitz-type serine protease inhibitor
LOAD  Late-onset Alzheimer’s disease
LPS  Lumboperitoneal shunt
LRP1  Low density lipoprotein receptor-related protein 1
MAPT  Microtubule-associated protein τ
MCI  Mild cognitive impairment
MMSE  Mini Mental State Examination
MRI  Magnetic resonance imaging
MS4A4E/6A  Membrane-spanning 4-domains, subfamily A, member 4E/6A
NCT  Nicastrin
NFT  Neurofibrillary tangle
NINCDS-ADRDA  National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association
NME8  NME/NM23 family member 8
PD  Parkinson’s disease
PEN-2  Presenilin enhancer 2
PICALM  Phosphatidylinositol binding clathrin assembly protein
PSEN 1/2  Presenilin 1/2
sAPPα/β  Soluble N-terminal products of APP processing
<table>
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<th>Abbreviation</th>
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<tr>
<td>SFWBT1</td>
<td>Scm-like with four MBT domains protein 1</td>
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<tr>
<td>SORL1</td>
<td>Sortillln-related receptor, L(DLR class) A repeats containing</td>
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<td>TREM2</td>
<td>Triggering receptor expressed on myeloid cells 2</td>
</tr>
<tr>
<td>TTR</td>
<td>Transthyretin</td>
</tr>
<tr>
<td>UTR</td>
<td>Untranslated region</td>
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<tr>
<td>VCI</td>
<td>Vascular cognitive impairment</td>
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<tr>
<td>VPS</td>
<td>Ventriculo-peritoneal shunt</td>
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1 Introduction

A syndrome of symptomatic hydrocephalus featuring normal or slightly elevated cerebrospinal fluid (CSF) pressure was first described in 1965 by Salomon Hakim and Raymond D. Adams, and they introduced the term “normal pressure hydrocephalus” (Hakim and Adams 1965). Although persistently elevated CSF pressure is not observed in this syndrome, the symptoms can be relieved by manipulating the CSF circulation with insertion of an implantable shunt device (Hakim and Adams 1965). In addition to Hakim's studies, some case reports of patients with clinical features and findings resembling this novel hydrocephalic syndrome had also been reported elsewhere (Foltz and Ward 1956, McHugh 1964).

This condition, still known as normal pressure hydrocephalus (NPH) is the most common form of hydrocephalic dementia. Typically, it displays a symptom triad including deterioration in gait, impaired cognition and urinary incontinence, with enlarged ventricles when viewed with brain imaging and normal or slightly elevated CSF pressure (Adams et al. 1965, Relkin et al. 2005). NPH can result from an earlier event, such as infection or trauma, in which case it is called secondary normal pressure hydrocephalus (Foltz and Ward 1956). When no predisposing factor or distinctive external cause can be determined, the condition is considered as idiopathic (iNPH)(Relkin et al. 2005). iNPH is a rare condition; its reported incidence varies between 0.5–6.3 cases with a prevalence in a range of 0.2–5.9 cases per 100 000 inhabitants a year (Tisell et al. 2005, Brean and Eide 2008, Klassen and Ahlskog 2011, Jaraj et al. 2014). In a Japanese study, the incidence in elderly people has been claimed even to be as high as 1.2 per 1000 inhabitants (Iseki et al. 2014).

The diagnostics of iNPH are hindered by the lack of knowledge of the underlying molecular causes of the disease and by the potential similarity in brain pathology with Alzheimer’s disease (AD), including the accumulation of amyloid-β (Aβ) and/or tau (τ) proteins (Leinonen et al. 2010, Leinonen et al. 2012b). iNPH is usually considered as a sporadic syndrome but there is some evidence for the existence of a familial form (Portenoy et al. 1984, Cusimano et al. 2011, McGirr and Cusimano 2012). This is reminiscent of the situation with AD where the sporadic type of the disease is more common but inheritable forms have also been described (Goate et al. 1991, Rogaev et al. 1995, Sherrington et al. 1995). In AD, inherited mutations affect the processing of amyloid precursor protein (APP), leading to an increase in the ratio of the more harmful, plaque forming isoforms of Aβ (Scheuner et al. 1996a). It has been debated whether AD and iNPH might share a genetic and/or molecular background due to the indicated similarity in their brain pathologies.

iNPH also seems to be a condition highly prone to many types of comorbidities (Malm et al. 2013). In fact, AD is an important differential diagnosis and a comorbidity for iNPH (Relkin et al. 2005). In the diagnostics, also other dementing conditions, especially Parkinson’s disease (PD) and vascular dementia, should be taken into consideration (Leinonen et al. 2010, Malm et al. 2013). The co-occurrence of iNPH with other dementing conditions is a diagnostic challenge and can affect the prognosis, but arterial hypertension has been found to be the most common comorbidity (Krauss et al. 1996b).

The heterogeneity of patient phenotypes and the relative obscurity with respect to any characteristic molecular biology mean that iNPH is even today a poorly understood syndrome. The disease prognosis and response to treatment is highly dependent on the successful recognition of patients with iNPH, since iNPH is often misdiagnosed. In this thesis, the main focus is placed on the molecular mechanism of Aβ production and the distinction between iNPH and AD. The aim is to increase knowledge of the pathophysiology on the genetic as well as at the protein level. Novel information might be
pivotal in achieving a better understanding of iNPH and consequently on designing more efficient, quality-of-life improving therapeutic solutions.
2 Review of the literature

2.1 IDIOPATHIC NORMAL PRESSURE HYDROCEPHALUS

Idiopathic normal pressure hydrocephalus (iNPH) is a rare, slowly progressive, dementing condition; its typical manifestations include gait difficulties, cognitive impairment and urinary incontinence in various combinations together with dilated brain ventricles and obliterated cortical sulci (Relkin et al. 2005). The underlying etiology of the condition is unknown. It affects the elderly population, arises without any apparent predisposing factors and presents itself via a disturbance in the CSF circulation without clearly elevating the intracranial pressure (ICP) (Hakim and Adams 1965, Relkin et al. 2005). Although this definition is widely used and accepted, there is no official and international scientific consensus for the classification of iNPH.

2.1.1 Clinical features

There are reports of some individuals with disease onset in their 40s or 50s but the mean age of iNPH onset is approximately 75 years and patients aged under 60 are very uncommon (Oi et al. 2000, Marmarou et al. 2005b). The definition of iNPH might seem straightforward but in reality, patients with suspected iNPH form a very heterogenous group with different combinations of clinical symptoms and various comorbidities (Mori 2001, Marmarou et al. 2005a, Malm et al. 2013). The most extensive clinical guidelines for iNPH diagnostics have been compiled by Japanese clinicians and their work represents the main foundation for this review (Mori et al. 2012).

The classic variations of gradually developed gait impairments in iNPH consist of a small-stepped gait, magnet gait and broad-based gait (Stolze et al. 2000, Stolze et al. 2001, Williams et al. 2008). Many terms have been used to describe the typical gait such as "apractic," "bradykinetic," "glue-footed," "magnetic," "parkinsonian," "short-stepped," and "shuffling" (Relkin et al. 2005). In the early stages, iNPH symptoms might be subtle, such as a difficulty in rising from a chair, weakness of the lower extremities, and fatigue brought on by walking (Relkin et al. 2005). When the disorder becomes more prominent, the patient takes shorter strides, walks slowly and unsteadily, especially when turning (Black 1980, Stolze et al. 2000, Marmarou et al. 2005b, Bugalho and Alves 2007, Klinge et al. 2012). The stride lengths might also vary and the foot rotation angles are increased (Stolze et al. 2000, Stolze et al. 2001). The gait might also feature freezing (Miyoshi et al. 2005). Unlike the situation in a well known extrapyramidal disease, PD, verbal commands, visual markings or such external cues seem to have little effect on gait difficulties (Stolze et al. 2001). After shunt surgery, an improvement in gait can be seen as lengthening of stride and better fluency during turning (Marmarou et al. 2005b, Bugalho and Alves 2007). The nature of these symptoms suggests that the impairment of gait in iNPH is derived from the lack of reciprocal coordination in the activation of different muscle groups. This would point to a disturbance in subcortical motor functions rather than a defect in the primary pyramidal tract (Relkin et al. 2005).

other hand, are better preserved (Nakayama et al. 2007). The patients are more oriented and perform better in memory tests than their AD counterparts (Miyoshi et al. 2005, Ogino et al. 2006). However, as the condition progresses, the impairment becomes more comprehensive, affecting all cognitive functions (Iddon et al. 1999). Shunt surgery has a positive effect on cognitive functions (Raftopoulos et al. 1994, Mataro et al. 2007) but improvement is dependent on both the type of cognitive decline and the severity of the symptoms (Thomas et al. 2005).

The third component of classic symptom triad is urinary incontinence. Urinary dysfunctions, i.e. incontinence and nocturnal micturition are quite common among the elderly and thus distinguishing these as iNPH related symptoms can be very difficult. iNPH patients typically suffer from overactive bladder and urgency-type urinary incontinence (Sakakibara et al. 2008). This can be seen in urodynamic measurements as a reduction of maximum flow rate and bladder capacity, and an increase in the residual volume (Sakakibara et al. 2008).

The accurate numbers of iNPH patients displaying each of these symptoms and the combinations are unknown. It is known that the gait difficulties are the most common, usually the first symptoms to appear and sometimes even the only symptoms. Gait difficulties develop in 91–100% of the patients (Mori 2001, Marmarou et al. 2005b, Relkin et al. 2005, Klinge et al. 2012). Cognitive impairment follows in 78–98% and urinary problems in 60–83%, with 50–60% of the patients exhibiting all three symptoms (Krauss et al. 1996b, Mori 2001, McGirt et al. 2005, Relkin et al. 2005, Factora and Luciano 2006, Hashimoto et al. 2010). As is typical for cognitive disorders, iNPH patients also suffer from various psychological symptoms, such as anxiety and apathy, depression and delusions (Kito et al. 2009).

2.1.2 Epidemiology

The iNPH is considered as a rare disease. The reported incidence varies between 0.5–6.3 cases with a prevalence ranging between 0.2–5.9 cases per 100,000 inhabitants each year (Tisell et al. 2005, Brean and Eide 2008, Klassen and Ahlskog 2011, Jaraj et al. 2014). In a Japanese study, the incidence in elderly people has been claimed even to be as high as 1.2 per 1000 inhabitants (Iseki et al. 2014). The problem with the prevalence and/or incidence studies of iNPH is that in many reports, the study population has been highly selected (i.e. patients in medical institutions) which most probably has influenced the results. The incidence of iNPH in terms of the whole population is most likely different. In studies considering patients in various medical centres, the incidence of iNPH has varied from 0.9–1.8 per 100,000 (Krauss and Halve 2004, Tisell et al. 2005, Brean et al. 2009, Klassen and Ahlskog 2011). In some studies, there has been also no differentiation between idiopathic and other forms of NPH (Vanneste et al. 1992, Trenkwalder et al. 1995), and thus the values should be considered with caution. In one Norwegian population based study, the incidence of iNPH was 5.5 per 100,000 and prevalence 21.9 per 100,000, these being the minimum estimates (Brean and Eide 2008). The same researchers later published data considering Norwegian patients shunted for iNPH and reported an incidence of approximately 1.09 per 100,000 (Brean et al. 2009). Their conclusion was that iNPH is a highly underdiagnosed condition. There is a clear paucity of proper population based studies investigating iNPH.

2.1.3 Pathophysiology

The pathophysiology of iNPH is diverse. There are studies which have detected inflammation of the arachnoid granulation (DeLand et al. 1972), thickening and fibrosis of the leptomeninges and arachnoid membrane (DeLand et al. 1972, Di Rocco et al. 1977, Bech et al. 1999), multiple infarcts due to arteriosclerotic and/or hypertensive vascular disease (Earnest et al. 1974, Di Rocco et al. 1977, Bech et al. 1999, Bech-Azeddine et al. 2007), ventricular ependymal disruption (DeLand et al. 1972, Di Rocco et al. 1977), and most
importantly, the pathological changes encountered in AD (Aβ plaques and/or neurofibrillary tangles) (Bech et al. 1999, Di Rocco et al. 1977, Bech-Azeddine et al. 2007, Golomb et al. 2000, Hamilton et al. 2010, Leinonen et al. 2010, Leinonen et al. 2012b). The pathological changes seem to vary from patient to patient and therefore no unequivocal pathological basis of iNPH has yet been established.

2.1.3.1 CSF circulation

There is some evidence for a disturbance in CSF resorption at arachnoid granulations or impaired CSF conductance through the subarachnoid space as the most probable reason for the ventricular dilation seen in iNPH (McGirt et al. 2005). In addition to this defect in CSF circulation, the symptoms have been considered to originate from ischemia in brain tissue, physical stress towards periventricular white matter, increased transmantle pressure and meningeal fibrosis (Fisher 1982, Conner et al. 1984, Ohata and Marmarou 1992, Waldemar et al. 1993, Uhli et al. 1999). Nonetheless, it has been proposed that the ventricular dilation may be independent of CSF malabsorption and is in fact secondary to a periventricular disease of the microvasculature, resulting in encephalomalacia and dilation of cerebral ventricles (Bradley et al. 1991). This hypothesis is supported by the association of iNPH with some other conditions affecting the vascular system, such as hypertension, reduced high-density lipoprotein cholesterol, ishemic heart disease, and diabetes (Earnest et al. 1974, Casmiro et al. 1989).

The CSF pressure measured as the opening pressure (CSF-OP) in lumbar puncture is within the range of 60–240 mm H2O; if the pressure lies from 105 mm H2O to 190 mm H2O, this is evidence for a diagnosis of probable iNPH in cases where all other diagnostic criteria have been met (Relkin et al. 2005). In non-iNPH controls, the CSF-OP averages 122 ± 34 mm H2O when measured from lumbar puncture (Bono et al. 2002).

2.1.3.2 Brain pathology

Most reports considering iNPH brain biopsy and post-mortem findings were conducted in the 1970s and 1980s and the number of iNPH cases was small. The patients also have suffered from significant co-morbidities such as vascular lesions (Heinz et al. 1970, Sohn et al. 1973, Earnest et al. 1974, Lorenzo et al. 1974, Vessal et al. 1974, Di Rocco et al. 1977, Koto et al. 1977, Ball and Vis 1978, Akai et al. 1987, Newton et al. 1989, Del Bigio et al. 1997). It has later been stated however, that so-called AD-related pathology - i.e. Aβ plaques - is frequently encountered in patients with suspected iNPH (Leinonen et al. 2010). In the case of induced hydrocephalus in rats, Aβ pathology has not been linked to disturbed CSF dynamics, since Aβ deposition did not significantly differ between elderly hydrocephalic rats and their age matched controls (Deren et al. 2009).

Two important aspartyl proteases are involved in APP processing and Aβ peptide formation, the β- (BACE1) and γ-secretases. It is well-established that AD patients display an approximately 30 % increase in the BACE1 levels and activity as compared to their age-matched, non-demented control subjects (Fukumoto et al. 2002, Yang et al. 2003, Ahmed et al. 2010). Conversely, no differences in γ-secretase activity have been detected between AD patients and non-demented subjects, although some qualitative alterations are thought to occur (Kakuda et al. 2012, Liu et al. 2013b). Recent studies have described an up-regulation of γ-secretase activity under hypoxic conditions and oxidative stress, which are considered to be both risk factors and clinical features of AD (Pluta 2007, de la Torre 2008, Pluta et al. 2009, Li et al. 2009b). In addition, iNPH symptoms are believed to be caused by impaired blood flow in the brain tissue surrounding the enlarged ventricles. Thus, ischemic and hypoxic stress conditions may have an effect on the neuropathology (Tabaton and Tamagno 2007). BACE1 is also a stress-related protease (Vassar et al. 2009), whose levels and activity are increased in certain pathological conditions, for example in hypoxia (Zhang et al. 2007) and ischemia (Wen et al. 2004). An increase in BACE1 activity has also been
shown to be directly linked to increased production of 40 and 42 amino acid Aβ (Aβ40 and Aβ42) both in vitro and in vivo (Sun et al. 2006).

2.2 DIAGNOSTICS OF iNPH

iNPH is routinely diagnosed based on examination, clinical features and brain imaging. It is a fact that a vast amount of test procedures have been developed to facilitate diagnostics and evaluation of clinical progression of iNPH (Relkin et al. 2005). Nevertheless, no single test has been found to be efficient and accurate enough to replace the combination of clinical assessment and neuroimaging in diagnostics (Relkin et al. 2005). These tests might improve diagnostic reliability, promote differential diagnostics or be valuable in evaluating shunt procedure outcome (Relkin et al. 2005).

2.2.1 Diagnostic criteria

The consideration that the patient has iNPH may arise from an incidental finding of vetriculomegaly from a brain imaging study or from his/her clinical symptoms (Relkin et al. 2005). One set of diagnostic criteria for iNPH were assembled in 2005 sub-dividing iNPH into probable, possible and unlikely categories (table 1) (Relkin et al. 2005). Furthermore, in 2012, it was suggested that in iNPH, ventricular dilatation seems to take place in the subarachnoid space in addition to the ventricles (Mori et al. 2012). Nonetheless, this dilatation phenomenon is not observed in some patients. Based on this finding, it has been postulated that iNPH can be also divided into two subgroups, disproportionately enlarged subarachnoid space hydrocephalus (DESH) and non-DESH (Mori et al. 2012). These findings have also verified that iNPH is a communicating form of hydrocephalus.

Supplemental tests can be used to assist in the diagnostics; these include neuropsychological testing, urodynamics, video- and computer-assisted gait assessment, functional brain imaging and other such procedures (Relkin et al. 2005).

The response of a patient suspected of suffering from iNPH to shunt placement has also been considered as a verification of the diagnosis i.e. a positive shunt response signifies iNPH (Ojemann et al. 1969). Nevertheless this assumption has been shown later as being incorrect since iNPH may progress to a stage where the response to shunt treatment is no longer significant (Marmarou et al. 2005b, Relkin et al. 2005). In addition, false-positive diagnoses occur because patients with similar conditions, such as secondary NPH and noncommunicating hydrocephalus, often respond favorably to shunt placement and also because placebo responses sometimes occur (Hebb and Cusimano 2001).
Table 1. Description of idiopathic normal pressure hydrocephalus classification: Probable, possible, and unlikely categories, adapted from Relkin et al. 2005.

<table>
<thead>
<tr>
<th>Probable iNPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>The diagnosis of probable iNPH is based on clinical history, brain imaging, physical findings, and physiological criteria.</td>
</tr>
</tbody>
</table>

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 months
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 48–72 h
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
Table 1. continues

III. Clinical

By classic definitions (Fisher 1977, Hakim and Adams 1965), etc., 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 Mini Mental State Examination), or evidence of at least two of the following on examination that are not fully attributable to other conditions

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

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

a. Episodic or persistent urinary incontinence not attributable to primary urological disorders
b. Persistent urinary incontinence
c. Urinary and fecal incontinence

Or any two of the following should be present

a. Urinary urgency as defined by frequent perception of a pressing need to void
b. Urinary frequency as defined by more than six voiding episodes in an average 12-hour period despite normal fluid intake
c. Nocturia as defined by the need to urinate more than two times in an average night
Table 1. continues

<table>
<thead>
<tr>
<th>IV. Physiological</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF opening pressure in the range of 5–18 mm Hg (or 70–245 mm H₂O) 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.</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Possible iNPH</th>
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</thead>
<tbody>
<tr>
<td>A diagnosis of possible iNPH is based on historical, brain imaging, and clinical and physiological criteria</td>
</tr>
</tbody>
</table>

I. History

Reported symptoms may

- a. Have a subacute or indeterminate mode of onset
- b. Begin at any age after childhood
- c. May have less than 3 months or indeterminate duration
- d. 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
- e. Coexist with other neurological, psychiatric, or general medical disorders but in the judgment of the clinician not be entirely attributable to these conditions
- f. Be nonprogressive or not clearly progressive

II. Brain imaging

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

- a. Evidence of cerebral atrophy of sufficient severity to potentially explain ventricular size
- b. Structural lesions that may influence ventricular size

III. Clinical

Symptoms of either

- a. Incontinence and/or cognitive impairment in the absence of an observable gait or balance disturbance
- b. Gait disturbance or dementia alone

IV. Physiological

Opening pressure measurement not available or pressure outside the range required for probable iNPH

<table>
<thead>
<tr>
<th>Unlikely iNPH</th>
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</thead>
<tbody>
<tr>
<td>1. No evidence of ventriculomegaly</td>
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<tr>
<td>2. Signs of increased intracranial pressure such as papilledema</td>
</tr>
<tr>
<td>3. No component of the clinical triad of iNPH is present</td>
</tr>
<tr>
<td>4. Symptoms explained by other causes (e.g. spinal stenosis)</td>
</tr>
</tbody>
</table>

Abbreviations: iNPH, idiopathic normal pressure hydrocephalus; CT, computed tomography; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; SPECT, single-photon emission computed tomography.
2.2.2 Neuroradiology

Ventriculomegaly on neuroimaging is one of the primary requisites for a diagnosis of hydrocephalus. Nonetheless, its extent can be subtle and its presence is not alone sufficient for diagnosis (Relkin et al. 2005, Mori et al. 2012). In addition to ventriculomegaly, broadened CSF spaces in the Sylvian fissures and basal cistern of iNPH patients when assessed by magnetic resonance imaging (MRI) volumetry have been reported (Kitagaki et al. 1998). Ventriculomegaly as visualized with cranial computer tomography (CT) and MRI can sometimes be misinterpreted as brain atrophy, and iNPH has been misdiagnosed as AD or some other neurodegenerative disease (Mori et al. 2012) (figure 1). One crucial differentiating feature is the tight high convexity of the subarachnoid spaces, which is seen in iNPH but not in AD (Kitagaki et al. 1998, Ishii et al. 2008a, Yamashita et al. 2010). The radiological criteria for iNPH include symmetrical quadri-ventricular enlargement with Evans’ index ≥ 0.3, which illustrates the increased ratio of ventricular size to cranial diameter (the ratio of the maximum width of the frontal horns to the maximum width of the inner table of cranium) (Caruso et al. 1997, Holodny et al. 1998, Kitagaki et al. 1998, Relkin et al. 2005, Sasaki et al. 2008, Klinge et al. 2012). The subarachnoidal spaces are either dilated or at least not narrowed in the Sylvian fissures and over the ventral surface below, and there is a narrowing in the spaces over the high cerebral convexity and media surface below (Kitagaki et al. 1998, Ishii et al. 2008b, Sasaki et al. 2008, Lee et al. 2010, Yamashita et al. 2010, Kojoukhova et al. 2015).

Usually CT is employed as an imaging method, since it is cheaper than MRI and suitable for patients with ferromagnetic implants or pacemakers. On the other hand, the advantages of MRI are its higher resolution, enabling more efficient evaluation of findings typical for iNPH and its better differential diagnostics (Relkin et al. 2005).

![Figure 1](image1.png)

*Figure 1. MRI image of AD brain (A) and iNPH brain (B). In AD, the brain atrophy can be seen throughout the cortex and hippocampi, whereas in iNPH findings such as narrowed calossal angle and broadened CSF spaces in the Sylvian fissure are typical.*
2.2.3 Neuropsychology

According to the diagnostic criteria of probable iNPH, the patient should present with at least two of the following symptoms if cognitive impairment is suspected: Psychomotor slowing (increased response latency), 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, or behavioral or personality changes (Relkin et al. 2005) (table 1). In addition, other psychiatric disorders have been encountered with iNPH, such as apathy, depression (Rosen and Swigar 1976, Price and Tucker 1977) and mania (bipolar disorder) (Schneider et al. 1996, Bekkelund et al. 1999).

Cognitive tests like Mini Mental State Examination (MMSE) are regularly applied in iNPH diagnostics, although MMSE is not very sensitive in detecting mild or moderate cognitive impairment, which is typically the case for early iNPH (Folstein et al. 1983). There is also a vast number of other neuropsychological tests which have been exploited: Grooved Pegboard, the Rey Auditory Verbal Learning Test, the Stroop Test, the Alzheimer's Disease Assessment Scale, the Wechsler Memory Scale -Revised, the Wechsler Adult Intelligence Scale -Revised, Rey-Osterrieth Complex Figure, Line-Tracing Test, Trail-Making Test, Boston Naming Test, Modified Token Test and Controlled Oral Word Association Test (Duinkerke et al. 2004, Thomas et al. 2005, Ogino et al. 2006, Hellstrom et al. 2012). These can be used in improving the reliability of the diagnosis and in resolving problems with the differential diagnostics.

2.2.4 Diagnosis of iNPH post-mortem

There are no validated post-mortem criteria for iNPH. The dilation of lateral and third ventricles might be detectable, as well as fibrous thickening of leptomeninges (Love 2005). At the microscopic level, the AD-like pathology, especially the presence of Aβ plaques is considered to be a frequent finding in iNPH brain (Leinonen et al. 2012b). In addition, large gaps in ependymal lining, gliosis in the periventricular region and ischaemic lesions in the deep white matter can be observed (Love 2005).

2.2.5 Differential diagnosis

Differential diagnosis of iNPH is rather challenging and the iNPH can be misdiagnosed as other neurodegenerative condition or cerebrovascular disease, such as AD, parkinsonian syndromes and vascular dementia (Leinonen et al. 2010, Magdalinou et al. 2013, Jingami et al. 2015) (Table 2). In addition, AD and vascular changes are also usually found as a comorbid condition in patients with iNPH (Malm et al. 2013).

iNPH is rather commonly mistakenly diagnosed as PD. The most typical symptom of iNPH is a gait impairment and thus iNPH cases can be readily misdiagnosed as PD, which is the most common movement disorder. The gait of iNPH might resemble the gait of a PD patient i.e. shortened length of step, apraxia and magnetism (Stolze et al. 2000, Stolze et al. 2001, Williams et al. 2008). An important differentiating feature is the fact that unlike in PD, visual markings, verbal commands or such assistance have only a marginal effect on the gait performance of the iNPH patient (Stolze et al. 2001). In addition to gait disturbances, other parkinsonian symptoms such as increased resting tone and prolonged reaction and movement times, and also difficulties in managing self-initiative tasks and executive dysfunction are typical findings in iNPH (Ogino et al. 2006, Chaudhry et al. 2007, Hellstrom et al. 2007, Mandir et al. 2007). Antiparkinsonian drugs such as levodopa do not exert any beneficial effects against the symptoms of iNPH (Mori et al. 2012).
<table>
<thead>
<tr>
<th>Table 2. Causes and characteristics of dementia (Modified from the report of Alzheimer’s association 2015).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alzheimer’s disease (AD)</strong></td>
</tr>
<tr>
<td>Most common cause of dementia; accounts for an estimated 60 percent to 80 percent of cases. About half of these cases involve solely AD pathology; many have evidence of pathologic changes related to other dementias.</td>
</tr>
<tr>
<td>Difficulty remembering recent conversations, names or events is often an early clinical symptom; apathy and depression are also often early symptoms. Later symptoms include impaired communication, disorientation, confusion, poor judgment, behavior changes and, ultimately, difficulty speaking, swallowing and walking.</td>
</tr>
<tr>
<td>It is recommended that AD is considered a slowly progressive brain disease that begins well before clinical symptoms emerge.</td>
</tr>
<tr>
<td>The hallmark pathologies of AD are the progressive accumulation of the protein fragment β-amyloid (plaques) outside neurons in the brain and twisted strands of the protein τ (tangles) inside neurons. These changes are eventually accompanied by the damage and death of neurons.</td>
</tr>
</tbody>
</table>

**Vascular dementia**

Previously known as multi-infarct or post-stroke dementia, vascular dementia is less common as a sole cause of dementia than AD, accounting for about 10 percent of dementia cases. However, it is very common in older individuals with dementia, with about 50 percent having pathologic evidence of vascular dementia (infarcts). In some cases, the infarcts coexist with AD pathology.

Impaired judgment or impaired ability to make decisions, plan or organize is more likely to be the initial symptom, as opposed to the memory loss often associated with the initial symptoms of AD.

Vascular dementia occurs most commonly from blood vessel blockage or damage leading to infarcts (strokes) or bleeding in the brain. The location, number and size of the brain injuries determine whether dementia will result and how the individual’s thinking and physical functioning will be affected.

In the past, evidence of vascular dementia was used to exclude a diagnosis of AD (and vice versa). That practice is no longer considered consistent with the pathologic evidence, which shows that the brain changes of AD and vascular dementia commonly coexist.

**Parkinson’s disease (PD) dementia**

Problems with movement (slowness, rigidity, tremor and changes in gait) are common symptoms of PD.

In PD, α-synuclein aggregates appear in an area deep in the brain called the substantia nigra. The aggregates are thought to cause degeneration of the nerve cells that produce dopamine.

The incidence of PD is about one-tenth that of Alzheimer’s disease.

As PD progresses, it often results in dementia secondary to the accumulation of Lewy bodies in the cortex or the accumulation of β-amyloid clumps and τ tangles (similar to Alzheimer’s disease).

**Normal pressure hydrocephalus (NPH)**

Symptoms include difficulty in walking, memory loss and inability to control urination.

Featured by impaired reabsorption of cerebrospinal fluid and the consequent build-up of fluid in the brain.

Can sometimes be corrected with surgical installation of a shunt in the brain to drain excess fluid.
AD is another important differential diagnosis when considering iNPH (Relkin et al. 2005). iNPH and AD resemble one another with regard to cognitive symptoms and also brain pathology. The typical and diagnostic clinical features of AD are the difficulties in learning and remembering new information, i.e. the impairment of episodic memory (86–94% of the patients) (Dubois et al. 2007, Dubois et al. 2014). In general, iNPH patients are more oriented than AD patients (Miyoshi et al. 2005, Ogino et al. 2006). CSF AD biomarkers may be used in differential diagnostics between iNPH and AD (Hall et al. 2012, Jeppsson et al. 2013, Jingami et al. 2015).

Neuroradiology is also very useful when differentiating iNPH from AD. Medial temporal lobe atrophy is typical and one of the diagnostic criteria for AD (Scheltens et al. 1992). In iNPH, there is tight high convexity in the subarachnoid spaces (Kitagaki et al. 1998, Ishii et al. 2008a, Yamashita et al. 2010). However, cortical and central atrophy is usually seen in moderate and severe stage of AD. In iNPH, neuroimaging reveals ventriculomegaly as one of the primary requisities, yet its presence alone is not sufficient for diagnosis (Relkin et al. 2005, Mori et al. 2012). In addition to ventriculomegaly, broadened CSF spaces in the Sylvian fissure and basal cistern of iNPH patients as MRI volumetry findings have been reported (Kitagaki et al. 1998). Neuroradiological features of iNPH have been discussed in more detail on section 2.2.2.

At the neuropathological level, AD and iNPH also seem to resemble one another as an accumulation of Aβ is present in both conditions (Braak and Braak 1991, Leinonen et al. 2010, Leinonen et al. 2012a). The hyper-phosphorylated τ (HPτ) tangles typical for AD are less specific in iNPH, and this needs to be considered in the differential diagnostics (Leinonen et al. 2010, Leinonen et al. 2012b). In fact, AD is more crucial as a comorbidity of iNPH than as a differential diagnosis and this will be further discussed on chapter 2.5 Comorbidities of iNPH.

Vascular cognitive impairment (VCI) and vascular dementia are heterogeneous syndromes associated with different types of cerebrovascular findings. With regard to the differential diagnostics of iNPH, subcortical vascular degeneration is the most important subtype of VCI, since its symptoms such as motor and cognitive dysexecutive slowing, urinary symptoms, and short-stepped gait bear a resemblance to iNPH (Roman et al. 2002, O’Brien et al. 2003, Relkin et al. 2005, Moorhouse and Rockwood 2008). In subcortical VCI, ischaemic deep white matter lesions can be viewed in the brain MRI. However, similar white matter changes are also nearly invariably encountered in patients with iNPH (Roman et al. 2002, Tullberg et al. 2002, O’Brien et al. 2003, Moorhouse and Rockwood 2008, Lenfeldt et al. 2011, Kojoukhova et al. 2015, Jaraj et al. 2016). Nevertheless in iNPH, there are typical neuroradiological findings, such as ventriculomegaly, broadened CSF spaces in the Sylvian fissure and basal cistern (Kitagaki et al. 1998, Ishii et al. 2008a, Yamashita et al. 2010), which can help in differentiating between these two conditions (discussed on section 2.2.2 Neuroradiology).

There are some other conditions that might exhibit symptoms similar to iNPH e.g. other hydrocephalic disorders, stroke, Huntington’s disease, frontotemporal dementia, infectious diseases affecting the central nervous system, urological disorders such as urinary tract infection and benign or malign prostatic enlargement, traumatic brain injury, depression and Wernicke’s encephalopathy (Bech-Azeddine et al. 2001, Relkin et al. 2005).
2.3 TREATMENT OF iNPH

iNPH has been treated by shunt surgery since its original description (Hakim and Adams 1965, Adams et al. 1965). The shunt consists of proximal and distal catheters, separated by a one-way valve. The shunt is typically inserted into lateral ventricle and distal catheter transports the excess CSF into the peritoneal cavity or right atrium of the heart, depending on the shunt type (Black 1980, Krauss et al. 1996a). The third option is a lumboperitoneal shunt (LPS), which takes CSF from the lumbar CSF space and transfers it to the peritoneal cavity (Spetzler et al. 1975, Kazui et al. 2015). The ventriculoperitoneal shunt (VPS) is the recommended treatment (Bergsneider et al. 2005). The efficiency of shunt treatment is highly dependent on the patient selection (Bergsneider et al. 2005, McGirt et al. 2005, Relkin et al. 2005, Eide and Sorteberg 2010). There is a paucity of level I evidence for the benefits of shunt surgery (Esmonde and Cooke 2002, Kazui et al. 2015), although there is some evidence that the majority of patients experience some improvement in their symptoms after shunting (Toma et al. 2013).

In addition to a stringently performed interview of patient and next-of-kin, supplemented by clinical evaluation and neuroradiological studies, there are some tests that can be performed when considering the suitability of a patient for undergoing a shunt procedure. For example, external lumbar drainage is considered a safe procedure with a high prediction value for identifying those iNPH patients who would most likely benefit from shunt surgery (Marmarou et al. 2005b). In addition, the CSF resistance testing and CSF tap test are considered as highly prognostic (Malm et al. 1995, Haan and Thomeer 1988, Walchenbach et al. 2002, Marmarou et al. 2005b, Wikkelso et al. 2013). An additional benefit associated with CSF resistance and tap testing is the possibility to perform these procedures in an outpatient setting (Marmarou et al. 2005b).

2.4 PROGNOSIS OF iNPH

2.4.1 Natural course

Rather few studies have investigated the natural course of iNPH and most of them have been flawed by selection bias, since it is not ethically permissible to conduct sham surgery, and furthermore, often the patient groups have not been homogenous (Savolainen et al. 2002, Scollato et al. 2008, Toma et al. 2011, Andren et al. 2014, Kazui et al. 2015). Symptoms of unshunted patients seem to deteriorate over time, although there is an extensive variability in the symptom progression at the level of the individual patient (Razay et al. 2009, Toma et al. 2011, Andren et al. 2014). Disease progression may vary from a slight improvement to a severe decline, although this is not invariably attributable to the characteristics of iNPH itself but may also be due to measurement errors, fluctuations in the patient's general condition, or the presence of comorbidities (Andren et al. 2014). Nonetheless, although it is rather difficult to estimate the prognosis for each single patient, the general trend is that if left untreated, his/her condition will deteriorate.

2.4.2 Outcome of treatment

Patients should be selected for shunting only if they fill the criteria for probable iNPH (ventriculomegaly shown with CT or MRI, two or more clinical symptoms of iNPH and the possibility of secondary causes excluded) (McGirt et al. 2005, Relkin et al. 2005, Williams and Malm 2016), and the diagnosis can be verified by using some additional test, i.e. 24 hour ICP monitoring, CSF-OP measurement or CSF tap test (Marmarou et al. 2005b, McGirt et al. 2005). It has also been shown that the patient may benefit from systematic follow-up after the procedure (McGirt et al. 2005). For example, the patient’s cognitive abilities and clinical status should be objectively evaluated, both the patient and his/her next-of-kin interviewed considering potential changes in symptom status and quality of life, preferably
evaluating each symptom individually. Thus the progression of possible comorbidities, shunt malfunctions and other issues can be addressed without delay (McGirt et al. 2005).

The first and most likely clinical response for CSF shunting is gait improvement, with dementia and urinary incontinence being less likely to improve after the operation (McGirt et al. 2005). In a large review of 64 studies, shunt surgery was followed by a positive outcome in an average of 71 % of patients with revision required for 16 % and complications encountered in 10.4 % (Toma et al. 2013). The long term beneficial outcome (minimum 3 years after surgery) was 65 % with an average of 53 % patients needing revision and a complication rate of 8.2 % (Toma et al. 2013).

The outcome of the shunt surgery is clearly dependent on accurate diagnostics and careful patient selection. The relief rate in clinical symptoms after surgery in patients diagnosed with purely clinical and radiological methods is generally considered to be 50 % (Relkin et al. 2005). The duration of symptoms and gait impairment as the primary symptom are independent predictors of outcome after CSF shunting for iNPH, as each additional year of symptom duration is associated with a 13 % lower likelihood of responding to shunting (McGirt et al. 2005). In addition, the typical MRI findings of iNPH are significant predictors of a positive shunt outcome (Virhammar et al. 2014). Additional tests, including the CSF pulse amplitude test, have improved the relief rate by up to 90 % (Eide and Sorteberg 2010).

2.4.3 Mortality and causes of death
iNPH patients are mostly elderly with a high frequency of cardiovascular comorbidities, and this is reflected in the causes of death i.e. cardio- and cerebrovascular diseases are the main causes of death, followed by injuries and malignant neoplasms (Black 1980, Malm et al. 2000, Spagnoli et al. 2006, Mirzayan et al. 2010, Leinonen et al. 2012a, Golz et al. 2014).

2.5 COMORBIDITIES OF iNPH
As described in a report by the International Society for Hydrocephalus and Cerebrospinal Fluid Disorders (ISHCSF), comorbidities encountered with iNPH patients include other degenerative brain diseases such as AD, PD and frontotemporal dementia, vascular diseases, musculoskeletal conditions, urinary problems, and psychiatric and behavioural disorders (Malm et al. 2013).

2.5.1 Concurrent degenerative brain disease
AD is one of the most common comorbidities encountered with iNPH (Relkin et al. 2005). Since iNPH and AD show similarities with regard to brain pathology, it can be difficult to distinguish between these two conditions (Leinonen et al. 2010, Leinonen et al. 2012b). The potential molecular similarity of these conditions has also led to a discussion of whether iNPH and AD would actually share the same pathophysiology and thus, at least to some extent, be manifestations of the same pathological processes (Silverberg et al. 2003). Cortical biopsies taken during shunt insertion have displayed AD pathology in 25–40 % of the cases (Golomb et al. 2000, Holm et al. 2003, Bech-Azeddine et al. 2007, Leinonen et al. 2012a). The higher occurrence of AD-like pathology in iNPH brain compared to the general population has also led to a proposal that there might be an AD-NPH syndrome (Cabral et al. 2011).

Prior to shunt surgery, it has been recommended to consider whether the iNPH truly is the main condition and the cause of the patient’s symptoms (Bech-Azeddine et al. 2007). For example, AD together with VCI has been associated with a poor response for shunt surgery. (Golomb et al. 2000, Bech-Azeddine et al. 2007, Hamilton et al. 2010, Leinonen et al. 2010, Cabral et al. 2011, Leinonen et al. 2012a, Koivisto et al. 2013).
2.5.2 Vascular risk factors and vascular disease
Hypertension, diabetes mellitus and a low serum level of high density lipoprotein cholesterol have been shown to be significant risk factors for iNPH in a few case-control studies (Jacobs 1977, Graff-Radford and Godersky 1987, Casmiro et al. 1989, Krauss et al. 1996b, Chang and Singh 2009, Jaraj et al. 2016). All of these are well known risk factors for vascular disease and thus it is logical that vascular changes coexist with iNPH (Casmiro et al. 1989, Mirzayan et al. 2010). High resistance to CSF outflow has been observed in iNPH patients; in one study where iNPH patients showed significantly greater frequency of retrograde jugular venous flow than control subjects while performing the Valsalva maneuver (Kuriyama et al. 2008). It has also been shown that iNPH patients display a higher incidence of glaucomatous disease compared to those without iNPH, suggesting the possibility of an increased neural susceptibility to pressure-related dysfunction underlying both diseases (Chang and Singh 2009).

It is not known whether vascular risk factors are an innate part of the pathophysiology of iNPH, contribute to the progression of the disease or simply co-occur without affecting the iNPH itself (Malm et al. 2013). Furthermore, it is not clear whether the symptoms of iNPH or the survival of the patients improves when these risk factors are treated. Cerebrovascular disease can be associated with irreversible brain damage and the disease process causes diverse metabolic changes in the brain (Schuff et al. 2003, Heiss and Zimmermann-Meinzingen 2012). It may be possible that multiple risk factors could act in synergy and worsen the prognosis compared to the situation when only a single factor is present (Malm et al. 2013).
2.6 ALZHEIMER’S DISEASE

2.6.1 Clinical features
AD is the most common dementia all around the world. According to the World Alzheimer Report prepared by Alzheimer’s Disease International (ADI) (Prince et al. 2015), the prevalence of AD dementia (%) for those aged 60 or more is 5.96–7.70 in Asia, 4.65–6.80 in Europe, 5.73 in United States of America, 8.34 in Latin America, and 4.36 for Sub-Saharan Africa. The numbers vary between sub-regions and the prevalence increases with age. It is also known that the development of AD is dependent on other factors e.g. environmental and socioeconomical factors and thus the differences in prevalence between geographic areas is to be expected (Kuller and Lopez 2011).

The typical and diagnostic clinical features of AD are difficulties in learning and remembering new information, i.e. the impairment of episodic memory (86–94 % of the patients) (Dubois et al. 2007, Dubois et al. 2014). Even at the early stage of the disease, other cognitive changes (executive dysfunction, impairments of complex visual processings and abstract thinking) are also present in addition to episodic memory impairment. (Dubois et al. 2007). A variety of neuropsychiatric symptoms such as apathy and delusions are also frequently encountered (Robert et al. 2005).

Mild cognitive impairment (MCI) is the term used to describe the state of individuals who have subjective memory and / or cognitive symptoms, objective memory and / or cognitive impairment, yet the activities of daily living are generally normal (Dubois et al. 2007). This term does not generally make any distinction between different potentially future-developing forms of dementia and thus the potentially preceding forms of any neurodegenerative memory or cognitive disorder can be referred to as MCI. One should also note that although MCI is a risk factor for progression to clinically diagnosed dementia, the conversion of MCI to dementia is not inevitable (Larrieu et al. 2002). In the case of a higher risk for AD, the term “amnestic cognitive impairment” has been proposed for individuals with subjective memory symptoms and objective memory impairment without notable difficulties in daily activities (Petersen et al. 1999). The reporting of subjective memory impairment is very common in the ageing population and might be due to multiple causes, including normal ageing, various medical conditions and depression (Dubois et al. 2007).

2.6.2 Diagnostics of Alzheimer’s disease
There are several different diagnostic criteria for AD; The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA ) and The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, American Psychiatric Assosiation 2000). Newer and improved diagnostic criteria include the International Working Group (IWG) for New Research Criteria for the Diagnosis of AD (Dubois et al. 2007) and IWG-2 (Dubois et al. 2014). These newer criteria take into account the findings of brain imaging and CSF biomarkers; their main goal is to achieve higher diagnostic accuracy and more efficient differential diagnoses.

The diagnostic criteria for AD are listed in table 3. The diagnosis is based on objective findings of a progressive cognitive decline, which is based by interviewing a next-of-kin and cognitive testing. The memory symptoms must start gradually and show a progressive decline over at least 6 months. Even very mild AD can be discriminated from normal healthy controls by tests of delayed recall with high accuracy (>90 %) (Welsh et al. 1991).

The medial temporal lobe atrophy is commonly present in AD. If one conducts a brain MRI on an suspected AD patient, then the atrophy in medial temporal lobe is often detected (71–96 % of the patients), whereas in MCI, it is seen with 59–78 % of the patients and only in 29 % of the normal aged subjects, and its extent correlates with disease severity (De Leon et al. 1997). AD-specific CSF biomarkers reflect the pathogenic processes taking place in the
brain tissue. A decreased CSF level of Aβ42 together with elevated levels of HPτ are typical findings in AD patients (Blennow et al. 1995, Motter et al. 1995, Mattsson et al. 2009).

Table 3. Diagnostic criteria for probable AD according to Dubois et al. 2007.

<table>
<thead>
<tr>
<th>Probable AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A and one or more supportive features B, C, D, or E</td>
</tr>
<tr>
<td>Core diagnostic criteria</td>
</tr>
<tr>
<td>A. Presence of an early and significant episodic memory impairment that includes the following features:</td>
</tr>
<tr>
<td>1. Gradual and progressive change in memory function reported by patients or informants over more than 6 months</td>
</tr>
<tr>
<td>2. Objective evidence of significantly impaired episodic memory on testing: this generally consists of recall deficit that does not improve significantly or does not normalize with cueing or recognition testing and after effective encoding of information has been previously controlled</td>
</tr>
<tr>
<td>3. The episodic memory impairment can be isolated or associated with other cognitive changes at the onset of AD or as AD advances</td>
</tr>
<tr>
<td>Supportive features</td>
</tr>
<tr>
<td>B. Presence of medial temporal lobe atrophy</td>
</tr>
<tr>
<td>Volume loss of hippocampi, entorhinal cortex, amygdala evidenced on MRI with qualitative ratings using visual scoring (referenced to well characterised population with age norms) or quantitative volumetry of regions of interest (referenced to well characterised population with age norms)</td>
</tr>
<tr>
<td>C. Abnormal cerebrospinal fluid biomarker</td>
</tr>
<tr>
<td>Low amyloid β1–42 concentrations, increased total τ concentrations, or increased Pτ concentrations, or combinations of the three</td>
</tr>
<tr>
<td>Other well validated markers to be discovered in the future</td>
</tr>
<tr>
<td>D. Specific pattern on functional neuroimaging with PET</td>
</tr>
<tr>
<td>Reduced glucose metabolism in bilateral temporal parietal regions</td>
</tr>
<tr>
<td>Other well validated ligands, including those that foreseeably will emerge such as Pittsburg compound B or FDDNP (2-{1-[6-(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl}-ethylidene)malononitrile</td>
</tr>
<tr>
<td>E. Proven AD autosomal dominant mutation within the immediate family</td>
</tr>
<tr>
<td>Exclusion criteria</td>
</tr>
<tr>
<td>History</td>
</tr>
<tr>
<td>Sudden onset</td>
</tr>
<tr>
<td>Early occurrence of the following symptoms: gait disturbances, seizures, behavioural changes</td>
</tr>
<tr>
<td>Clinical features</td>
</tr>
<tr>
<td>Focal neurological features including hemiparesis, sensory loss, visual field deficits</td>
</tr>
<tr>
<td>Early extrapyramidal signs</td>
</tr>
</tbody>
</table>
Table 3. continues

<table>
<thead>
<tr>
<th>Other medical disorders severe enough to account for memory and related symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-AD dementia</td>
</tr>
<tr>
<td>Major depression</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td>Toxic and metabolic abnormalities, all of which may require specific investigations</td>
</tr>
<tr>
<td>MRI FLAIR or T2 signal abnormalities in the medial temporal lobe that are consistent with infectious or vascular insults</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Criteria for definite AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both clinical and histopathological (brain biopsy or autopsy) evidence of the disease, as required by the NIA-Reagan criteria for the post-mortem diagnosis of AD; criteria must both be present</td>
</tr>
<tr>
<td>Both clinical and genetic evidence (mutation on chromosome 1, 14, or 21) of AD; criteria must both be present</td>
</tr>
</tbody>
</table>

2.6.3 Pathophysiology

One of the hallmarks of AD is the accumulation of Aβ and HPτ (Braak and Braak 1991, Braak et al. 2006). However, the accumulation of Aβ and HPτ is also a normal age dependent phenomenon and the presence of Aβ and HPτ is not strictly related to cognitive impairment (Braak and Braak 1997, Ingelsson et al. 2004) but especially in younger subjects, the association of this pathology with dementia is stronger than in their more elderly counterparts (Savva et al. 2009). The accumulation of Aβ and HPτ is detectable in the medial temporal structures (hippocampal formation, parahippocampal gyrus, entorhinal cortex) and it usually begins even years before the onset of clinical symptoms (Braak and Braak 1991). Nonetheless, the absence of both Aβ and HPτ in frontal cortical brain biopsy does predict a low probability for later development of AD (Leinonen et al. 2010).

Aβ is derived from APP by several proteolytic cleavages (Selkoe 1998). APP processing starts with either predominant, non-amyloidogenic cleavage by α-secretase (Asai et al. 2003, Kuhn et al. 2010) within the Aβ region, or amyloidogenic BACE1 cleavage at the amino terminus of the Aβ domain. Another enzyme, γ-secretase, is responsible for the further processing of carboxy-terminal fragments (De Strooper et al. 1998, Francis et al. 2002, Ed Bauer et al. 2003). The processing of APP is discussed in greater detail in chapter 2.8. Molecular mechanism of the processing of Aβ precursor protein.

2.6.3.1 τ pathology

τ is a protein encoded by the microtubule-associated protein τ (MAPT) gene with its expression varying with regard to developmental stage of the individual (Goedert et al. 1989). Six isoforms are produced by alternative splicing and the transcripts are predominantly produced by neurons throughout the adult human nervous system (Goedert et al. 1989). τ is located mainly in the axons where it functions as a microtubule binding protein with a role in microtubule stability and assembly. The ability of τ to bind microtubules is dependent on phosphorylation and dephosphorylation which alter its conformation (Brandt et al. 2005, Iqbal et al. 2009, Dolan and Johnson 2010). In AD, the accumulation of τ and the formation of neurofibrillary tangles (NFTs) have been claimed to be a crucial hallmark (Wood et al. 1986). The severity of AD correlates with the distribution of NFTs within medial temporal cortex (Guillozet et al. 2003).
Due to function of τ as a microtubule associated protein, it has been debated whether the pathogenic effects of τ in AD might be mediated by defects in microtubule integrity (Dolan and Johnson 2010). The prevailing model of microtubule-related τ toxicity indicates that phosphorylation of τ precedes the dissociation of the protein from microtubules, and this event is followed by the aggregation of phosphorylated τ, leading to NFT formation in AD brains (Iqbal et al. 2009, Dolan and Johnson 2010). There is no demarcation line between the normal, physiological rates of phosphorylation and dephosphorylation of τ required for microtubule stability and neuronal health, and the disturbances leading to the pathogenic cascade.

Defective axonal transport has been considered to play a part in several neurodegenerative disorders such as AD (Stokin et al. 2005) and thus it has been postulated that some pathological effects of τ might arise from its role in axonal transport. Indeed, impairment of axonal transport seems to be followed by increase in Aβ levels and these reductions in microtubule-dependent transport may promote the pathological processing of APP (Stokin et al. 2005, Dolan and Johnson 2010). Importantly, τ pathology is not seen only in AD but is also manifested in various other neurodegenerative disorders, collectively called taupathies (Hutton et al. 1998).

τ is also linked to the Aβ pathology but the specific mechanism is not known. It is far from clear whether the accumulation of HPτ or Aβ comes first and if these events take place independently of one another. Since in familial Alzheimer’s disease (FAD), mutations of APP gene are sufficient to result in Aβ deposition and the formation of τ tangles, it has been hypothesized that Aβ accumulation precedes HPτ (Selkoe and Hardy 2016). Nevertheless there have been various reports pointing to a reciprocal relationship between Aβ and τ. It seems that soluble Aβ oligomers can induce τ phosphorylation both in vitro and in vivo (Deshpande et al. 2006, Johansson et al. 2006, Chabrier et al. 2012, Choi et al. 2014, Lloret et al. 2015) and on the other hand, τ would be necessary in mediating the toxic effects of Aβ (Takashima et al. 1993, Stokin et al. 2005).
2.7 GENETIC BACKGROUND OF iNPH AND AD

2.7.1 Causative mutations
AD can be divided into two subgroups, early onset (EOAD, or familial FAD) and late onset (LOAD) based on the onset age which is under 65 in EOAD and 65 or more in LOAD. Approximately 90% of the patients with AD have the LOAD form, since the autosomal dominant mutations causing EOAD are rare. There is also a genetic component in LOAD since it displays an estimated 59–78% heritability (Gatz et al. 2006) but the genetics are more complex and not as highly penetrable as the mutations in APP, presenilin 1 gene (PSEN1) and presenilin 2 gene (PSEN2) encountered in EOAD.

To date 49 mutations in APP, 215 in PSEN1 and 13 in PSEN2 have been claimed to cause EOAD (Cruts et al. 2012). Nonetheless, one needs to bear in mind that autosomal dominant mutations account for only 1% of AD cases and in EOAD 20–70% of cases are estimated to have a PSEN1 mutation, 10–15% of cases are estimated to have an APP mutation, and PSEN2 mutations are rare (Bekris et al. 2010). All these genes exert direct effects on the processing of APP in a manner where the concentration of the more amyloidogenic Aβ42 is increased at the expense of the Aβ40 form (Scheuner et al. 1996b).

The potential genetic background for iNPH has been intensely studied and some indications of possible genetic component have been found. A few rare cases have been reported, where siblings have clinical features indistinguishable from iNPH (Portenoy et al. 1984, Cusimano et al. 2011). Another study described a large family of individuals in three generations with clinical and MRI features typical for iNPH (Takahashi et al. 2011). At present, only copy number variations in the SFMBT1 gene (Scm-like with four MBT domains protein 1) have been shown to be more common in individuals with the iNPH-like ventriculomegaly encountered in MRI in comparison with normal individuals (Kato et al. 2011).

2.7.2 Apolipoprotein E ε4
Apolipoprotein E (ApoE) is a lipid transporter present in the cells of different organs and tissues (Mahley 1988, Huang and Mahley 2014). In brain, ApoE is produced mainly by astrocytes (Huang and Mahley 2014). It is the major transporter of lipids, and it also binds Aβ (Bu 2009). Its production is induced after a peripheral nerve injury and it appears to have a crucial role in neuronal repair by delivering lipids to regenerating axons and Schwann cells (Kim et al. 2014). There are three different ApoE isoproteins coded by three APOE alleles; ε2, ε3, and ε4 (Zannis et al. 1982). ApoE3 is the common form; ApoE2 and ApoE4 differ from it at positions 112 or 158 by a single amino acid, either cysteine or arginine (Singh et al. 2010, Kim et al. 2014). The ApoE isoforms regulate Aβ aggregation and clearance in the brain via proteolytic degradation of Aβ (Jiang et al. 2008). There are isoform specific differences in binding to Aβ, i.e ApoE4 has a poorer ability to decrease Aβ clearance in mice compared to ApoE3 (Castellano et al. 2011).

Many family studies and genetic analyses have revealed that the ε4 allele of the APOE is a major risk factor for LOAD, both lowering the disease onset age and increasing its occurrence (Corder et al. 1993, Bu 2009, Huang and Mucke 2012). In diploid genome, each individual carries two alleles of each gene and in the case of the APOE having one copy of ε4 allele triples the risk for AD and the risk is 15-fold higher in carriers of two copies (Farrer et al. 1997). The frequency of the ε4 allele is high in patients with AD, being encountered in approximately 40% of the patients (Farrer et al. 1997). Thus it seems that individuals carrying the ε4 allele are at higher risk of developing AD compared to individuals with ε3 allele, and ε2 even appears to decrease the risk (Kim et al. 2014).

In addition to directly Aβ linked functions, ApoE has been found to have a role in regulating brain lipid transport, glucose metabolism, neuronal signaling, neuroinflammation and mitochondrial function, possibly also contributing to the AD pathogenesis via these routes (Chen et al. 2010, Liu et al. 2013a). Although there have been
many studies attempting to elucidate the underlying mechanism with higher precision, the manner in which ApoE4 influences AD onset and progression has yet to be revealed.

2.7.3 Other potential genetic risk factors
The search for LOAD genes has been intensive, yet the APOE ε4 allele remains the only firmly established genetic susceptibility factor. It seems that the APP processing is not as crucial to the pathogenesis of LOAD as it is in EOAD (Medway and Morgan 2014). Nevertheless, thanks to the Genome Wide Association Studies (GWAS) nearly 1000 papers considering the potential association between reputed risk alleles of different genes and AD have been published (Bertram et al. 2007). Because of this ever-multiplying pool of information, a need for systematic meta-analysis and the necessity of developing some way to keep track of the findings, it was decided to establish the Alzgene database (Bertram et al. 2007). The ten most studied AD-risk genes according to the AlzGene database have been APOE, BIN1, CLU, ABCA7, CR1, PICALM, MS4A6A, CD33, MS4A4E, and CD2AP, with an additional 12 recently identified genes being FRMD4A, INPP5D, HLA_DRB5, NME8, EPHA1, PTK2B, CELF1, SORL1, FERMT2, SLC24A4, DSG2 and CASS4 (Lambert et al. 2009, Lambert et al. 2013a, Lambert et al. 2013b). These genes and their relationship with AD are presented in table 4.
### Table 4. AlzGene top 10 and novel AD risk genes.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene / protein name</th>
<th>Relevance to Alzheimer’s disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AlzGene top 10</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
<td>A major predisposing gene associated with both familial and sporadic AD, increasing the occurrence and lowering the age of onset of the disease.</td>
<td>(Corder et al. 1993, Roses 1996, Bu 2009, Huang and Mahley 2014)</td>
</tr>
<tr>
<td>BIN1</td>
<td>Bridging integrator 1</td>
<td>Associated with neuronal degeneration. SNPs within BIN1 locus (rs744373 and rs7561528) have been found to increase the risk of AD. Specific role of these SNPs is unknown.</td>
<td>(Wang et al. 2016)</td>
</tr>
<tr>
<td>CLU</td>
<td>Clusterin / Apolipoprotein J</td>
<td>Affects the Aβ aggregation in a substrate quantity-dependent manner, either preventing or promoting aggregation.</td>
<td>(Wilson et al. 2008, Beeg et al. 2016)</td>
</tr>
<tr>
<td>ABCA7</td>
<td>ATP-binding cassette, subfamily A, member 7</td>
<td>ABCA7 deficiency has been associated with accumulation of Aβ-plaques possibly via BACE1 mediated effects, although no alterations in clearance rate have been detected. Possibly associates with cortical and hippocampal atrophy.</td>
<td>(Sakae et al. 2016, Ramirez et al. 2016)</td>
</tr>
<tr>
<td>CR1</td>
<td>Complement receptor 1</td>
<td>Associated with phagocytic clearance of Aβ in the blood circulation.</td>
<td>(Rogers et al. 2006)</td>
</tr>
<tr>
<td>PICALM</td>
<td>Phosphatidylinositol-binding clathrin assembly protein</td>
<td>PICALM is able to rescue the Aβ-induced deficits in clathrin-mediated endocytosis. Involved in clathrin-mediated endocytosis of γ-secretase. In addition, the potential role in the clearance of Aβ across the BBB.</td>
<td>(Treusch et al. 2011, Kanatsu et al. 2014, Zhao et al. 2015)</td>
</tr>
<tr>
<td>CD33</td>
<td>CD33 molecule/ myeloid cell surface antigen CD33</td>
<td>Increased expression of microglia-associated CD33 seems to result in increasing AD pathology, but there has also been some evidence that the effects on Aβ accumulation are splice variant dependent and can be either pro- or anti-accumulative.</td>
<td>(Griciuc et al. 2013, Walker et al. 2015)</td>
</tr>
<tr>
<td>CD2AP</td>
<td>CD2 associated protein</td>
<td>SNPs rs9296559, rs9349407, and rs10948363 are associated with increased AD risk but specific functions are unclear. SNP rs9349407 associates with neuritic plaque burden in AD brains.</td>
<td>(Shulman et al. 2013)</td>
</tr>
</tbody>
</table>
**Table 4. continues**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Associated SNPs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS4A6A</td>
<td>Membrane-spanning 4-domains, subfamily A, member 6A</td>
<td>SNPs associated with AD, rs610932 (MS4A6A), rs670139 (MS4A6A and MS4A4E) and rs983392 (MS4A6A).</td>
<td>(Hollingworth et al. 2011, Ramirez et al. 2016)</td>
</tr>
<tr>
<td>MS4A4E</td>
<td>Membrane-spanning 4-domains, subfamily A, member 4E</td>
<td>Specific function unknown. MS4A6A associates potentially with cortical and hippocampal atrophy.</td>
<td>(Lambert et al. 2009, Lambert et al. 2013a, Lambert et al. 2013b)</td>
</tr>
<tr>
<td>FRMD4A</td>
<td>FERM domain containing 4A</td>
<td>Function in AD pathogenesis is potentially related to modulation of τ pathology.</td>
<td>(Yan et al. 2016)</td>
</tr>
<tr>
<td>INPP5D</td>
<td>Inositol polyphosphate-5-phosphatase</td>
<td>Potentially associated with increased permeability of the BBB. Also binds CD2AP in plasmacytoid dendritic cells.</td>
<td>(Bao et al. 2012, Jickling et al. 2013)</td>
</tr>
<tr>
<td>HLA_DRB5</td>
<td>Major histocompatibility complex, class II, DR beta 5</td>
<td>Activates microglia, knockout is potentially protective against neurodegeneration caused by Aβ and HPτ accumulation. DNA methylation changes within HLA_DRB5 locus are associated with Aβ load and τ tangle density.</td>
<td>(Rosenthal and Kamboh 2014, Yu et al. 2015)</td>
</tr>
<tr>
<td>NME8</td>
<td>NME/NM23 family member 8</td>
<td>Regulates oxidative stress levels which potentially links NME8 to AD pathogenesis. The genotype of SNP rs2718058 associates with CSF Pτ levels and neuroimaging traits such as occipital and hippocampal atrophy.</td>
<td>(Rosenthal and Kamboh 2014, Liu et al. 2014)</td>
</tr>
<tr>
<td>EPHA1</td>
<td>EPH receptor A1</td>
<td>SNPs within EPHA1 correlate with increased rate of memory decline and associate with dementia progression. SNPs also correlate with atrophy of occipitotemporal gyrus, inferior temporal gyrus and hippocampus.</td>
<td>(Carrasquillo et al. 2015, Wang et al. 2015)</td>
</tr>
<tr>
<td>PTK2B</td>
<td>Protein tyrosine kinase 2 beta</td>
<td>PTK2B is possibly involved in dendritic shrinkage in the hippocampus via chronic stress.</td>
<td>(Kinoshita et al. 2014)</td>
</tr>
<tr>
<td>CELF1</td>
<td>CUGBP, Elav-like family member 1</td>
<td>SNP rs10838725 within the CELF1 locus confers a risk effect for AD. Possibly is also a modulator of τ toxicity.</td>
<td>(Shulman et al. 2014)</td>
</tr>
</tbody>
</table>
Table 4. continues

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Association</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SORL1</td>
<td>Sortilin-related receptor, L(DLR class) A repeats containing</td>
<td>SORL1 is a receptor directing APP for recycling in the endocytic pathway. Within the SORL1 locus, the methylation status of certain CpG sites has been associated with Aβ load, tangle density, and pathological AD diagnosis.</td>
<td>(Rogaeva et al. 2007, Yu et al. 2015)</td>
</tr>
<tr>
<td>FERMT2</td>
<td>Fermitin family member 2</td>
<td>Potentially related to τ pathology.</td>
<td>(Shulman et al. 2014)</td>
</tr>
<tr>
<td>SLC24A4</td>
<td>Solute carrier family 24, member 4</td>
<td>Within SLC24A4, the methylation status of CpG dinucleotides has been associated with Aβ load and pathological diagnosis of AD.</td>
<td>(Yu et al. 2015)</td>
</tr>
<tr>
<td>DSG2</td>
<td>Desmoglein 2</td>
<td>SNP rs8093731 was associated positively in a meta-analysis with AD risk, yet this association was not seen later.</td>
<td>(Lambert et al. 2013b)</td>
</tr>
<tr>
<td>CASS4</td>
<td>Cas scaffolding protein family member 4</td>
<td>CASS4 might interact with PTK2B. SNPs within CASS4 associated with AD and AD-related neuropathological features. Specific effects are unknown.</td>
<td>(Singh et al. 2008, Lambert et al. 2013b, Beecham et al. 2014)</td>
</tr>
</tbody>
</table>
2.8 MOLECULAR MECHANISM OF THE PROCESSING OF AMYLOID PRECURSOR PROTEIN

2.8.1 Aβ production
Aβ is derived from APP after several proteolytic cleavages (Selkoe 1998) (figure 2). The full-length APP is axonally transported to both peripheral and central synapses, implying a central role in the regulation of presynaptic structure and function (Buxbaum et al. 1998). In addition, full-length APP could function as a cell surface G-protein-coupled receptor and it can be phosphorylated and thus released from cells, and it is known to undergo constitutive endoproteolysis within a transmembrane domain (Haass et al. 1992, Shoji et al. 1992, Okamoto et al. 1995, Selkoe 1998). Furthermore, the fragments resulting from APP processing all possess a variety of properties including cell adhesion, neurotrophic and neuronal proliferative activity, intercellular communication and membrane-to-nucleus signaling (Turner et al. 2003). The APP gene spans almost 300 000 bases of DNA from which 18 exons can be alternatively spliced (Hattori et al. 1997). A functional classification can be made subdividing those APP isoforms containing exon 7 that codes for a Kunitz-type serine protease inhibitor (KPI) domain (751, 770, amyloid precursor related protein (APRP) 365 and APRP563), and those without (695 and 714,) (Preece et al. 2004). The 695-residue isoform is the primary form produced by neurons (Selkoe 1998). It has been indicated that in sporadic AD, some qualitative changes favoring the production of 751- and 770-isoforms exist, with the overall quantity of the APP expression remaining unchanged compared to non-demented controls (Preece et al. 2004, Matsui et al. 2007, Martiskainen et al. 2015b).

APP processing starts with either predominant, non-amyloidogenic cleavage by α-secretase (Asai et al. 2003, Kuhn et al. 2010) within the Aβ region, or amyloidogenic BACE1 cleavage at the amino terminus of the Aβ domain. The cleavage by α-secretase leads to the formation of a C-terminal C83 fragment in addition to a soluble APPα fragment. In turn, cleavage of APP by BACE1 evokes the formation of membrane bound, C-terminal fragment C99 and soluble APPβ fragment, which is released into endosomal or trans-Golgi lumen (Vassar et al. 1999). It has been considered that the cellular APP levels are not the rate limiting factor for these cleavages and thus a higher activity of one form of enzymatic cleavage would not decrease the amount of the others (Postina et al. 2004). It has also been proposed that the α-secretase and BACE1 would compete for the substrate APP, and have opposite effects on Aβ generation by cleaving APP at different sites (Selkoe and Schenk 2003, Fahrenholz 2007, Kuhn et al. 2010). In fact, elevated BACE1 activity has been shown to decrease α-secretase activity (Vassar et al. 1999, Kuhn et al. 2010).

BACE1 has its main cleavage site at the N-terminus of the Aβ-sequence and a secondary site within the Aβ sequence (Vassar et al. 1999), whereas α-secretase cleaves only within the Aβ-domain, thus decreasing Aβ production (Esch et al. 1990, Kuhn et al. 2010). The soluble product of α-cleavage has also been shown to exert neuroprotective properties in a study conducted in mice (Ring et al. 2007). ADAM10 has been determined to be the primary enzyme involved in α-secretase cleavage (Kuhn et al. 2010). It is well-established that AD patients show an approximately 30 % increase in BACE1 levels and activity as compared to age-matched, non-demented subjects (Fukumoto et al. 2002, Yang et al. 2003, Ahmed et al. 2010).

The γ-secretase is responsible for the further processing of carboxy-terminal C83 and C99 fragments. The γ-secretase is an intramembrane protease consisting of four different subunits, which are catalytic aspartyl protease presenilin 1 or 2 (PSEN 1/2), anterior pharynx-defective 1 (APH-1), nicastrin (NCT) and presenilin enhancer 2 (PEN-2) (De Strooper et al. 1998, Francis et al. 2002, Edbauer et al. 2003). The specific function of the subunits other than presenilins and their contribution to γ-secretase are poorly understood, although it is known that these four components are both sufficient and necessary for γ-secretase function (Edbauer et al. 2003). There might still be additional proteins which have
regulatory functions, even though they are not integral parts of the γ-secretase (Li et al. 2009a).

The γ-secretase cleavage of both C83 and C99 produces an APP intracellular domain (AICD) and an extracellular p3 from C83 and the Aβ peptides of different lengths from C99, varying between 37–43 amino acids. The Aβ42 and Aβ40 are the most prevalent isoforms; Aβ42 is the main component of Aβ plaques seen in neuropathological conditions such as AD and iNPH, and it has been shown to be more prone to oligomerization and aggregation than its Aβ40 counterpart (Jarrett et al. 1993, Hardy and Selkoe 2002). Although the quantitative activity of γ-secretase is not altered in AD, some qualitative changes favouring the Aβ42 form might occur (Kakuda et al. 2012). The γ-secretase is also crucial for many other cellular processes, for example in the Notch signaling pathway (Haapasalo and Kovacs 2011). Thus it has been claimed that it would not be therapeutically beneficial to disturb the normal activity of the secretase (De Strooper et al. 1999).

Figure 2. Amyloid precursor protein (APP) processing. sAPPα/β = soluble APPα/β, α = α-secretase, β = β-secretase (BACE1), γ = γ-secretase, AICD = APP intracellular domain, Aβ = amyloid β. Figure modified from Nicolas and Hassan 2014.
2.8.2 Aβ clearance
Aβ clearance occurs either via transportation of intact Aβ across the blood-brain barrier (BBB) or degradation within the brain. The BBB separates the central nervous system (CNS) from circulating blood, thus it is important in maintaining tissue homeostasis by regulating the exchange of substances between the bloodstream and CNS tissue. Endothelial cells of the brain capillaries are the main cell type contributing to the formation of BBB (Abbott et al. 2006). The low density lipoprotein receptor-related protein 1 (LRP1) has been postulated to act as a potential mediator transporting Aβ across the BBB (Shibata et al. 2000, Pflanzner et al. 2011, Sagare et al. 2012). On the other hand, there have been suggestions that LRP1 alone would not be able to mediate the transport and some co-transporter on the side of endothelial cell facing the capillary lumen would be required (Krieger and Herz 1994, Hartz et al. 2010). It has been speculated that LRP1 is the main mediator for the uptake of Aβ into neurons, binding Aβ42 either directly or via Aβ chaperones, such as ApoE (Bu et al. 2006, Yamada et al. 2008, Fuentealba et al. 2010). Aβ is transported into lysosomes where it is degraded. However, if the lysosomal concentration exceeds the lysosomal degradation capacity, Aβ42 starts to accumulate and then aggregates, and this is toxic to the cell (Ji et al. 2002, Ji et al. 2006, Fuentealba et al. 2010). This toxicity seems to be enhanced in the presence of ApoE4 (Ji et al. 2002, Ji et al. 2006).

LRP1 has also a role in the so-called peripheral sink hypothesis of Aβ. In plasma, the soluble form of LRP1 (sLRP) is the major Aβ binding protein, with 70% of Aβ42 and 90% of Aβ40 bound to this protein under normal conditions (Sagare et al. 2007). In patients with AD, the amount of sLRP bound Aβ40 and Aβ42 declines with a simultaneous increase in free Aβ40 and Aβ42 (Sagare et al. 2007). This is significant since the Aβ that is bound to sLRP cannot be taken back up by the brain and instead is broken down in the liver.

LRP1 might also have a role in the processing of APP, thus possibly regulating the formation of Aβ42 (Waldron et al. 2008). LRP1 and APP have been shown to interact and there are some results indicating that the endocytic trafficking of APP might be affected by the rapid endocytosis of LRP1 (Cam et al. 2005, Bu et al. 2006). The relevance of LRP1 in APP processing in AD has however been challenged, since significantly decreased levels of LRP1 have been seen in the brain not only of AD patients, but also in elderly people in general (Kang et al. 2000, Shibata et al. 2000). Thus it is possible that LRP1 might not contribute to the pathological processing of APP but it still may have an important role in Aβ clearance.

In addition to LRP1 mediated clearance, Aβ can be engulfed by microglia cells if it is present in its soluble form (sAβ) (Mandrekar et al. 2009). Microglia can also phagocytose fibrillary Aβ, but degradation of Aβ is only possible when the cells are activated, since only these types of cells have sufficient acidity in their lysosomes (Majumdar et al. 2007). Several other enzymes are also able to degrade soluble Aβ; the most important one in the extracellular space is the insulin-degrading enzyme (Qiu et al. 1998) and intracellularly neprilysin (Iwata et al. 2000).

Microglia and astrocytes are involved in a process called neuroinflammation, which is an essential feature in AD pathology (Haga et al. 1989, Mrak et al. 1996). It has been postulated that the tissue damage and the presence of material such as Aβ plaques and HPτ tangles would serve as a main trigger for an inflammatory reaction (Akiyama et al. 2000). Microglia start to phagocytose Aβ fibrils via receptor ligation (Heneka et al. 2015). As a result, there is a persistent functional impairment of microglial cells at plaque sites (Streit et al. 2009, Krabbe et al. 2013). In addition to microglia, hypertrophic reactive astrocytes accumulate in the proximity of senile plaques; these cells can be detected in post-mortem tissue from AD patients (Medeiros and LaFerla 2013).
2.8.3 Transthyretin and Aβ

Transthyretin (TTR) is a 55 kDa homotetrameric protein involved in the transport of thyroid hormones and retinol. TTR has been considered to be the main Aβ binding protein in CSF and it has been found co-localized in hippocampal Aβ plaques and on the blood vessels of AD patients (Schwarzman et al. 1994, Schwarzman and Goldgaber 1996). Moreover, the anti-TTR serum has been shown to stain the majority of neuronal bodies in AD brains but only 10% of neurons in age-matched non-demented controls (Li et al. 2011). This increased staining has also been proven to be due to an increase in the actual synthesis of TTR (Li et al. 2011). According to studies performed on APP23 transgenic mice, TTR exerted a considerable protective effect on both the formation of Aβ deposition and the neuronal effects caused by toxic Aβ species (Buxbaum et al. 2008). TTR seems to bind Aβ and thus hinder plaque formation and prevent toxicity possibly by cleaving Aβ (1−42) (Liu and Murphy 2006, Costa et al. 2008a). TTR also seems to be able to disrupt preformed Aβ fibers and it displays a proteolytic activity on aggregated Aβ (Costa et al. 2008b, Costa et al. 2008a). TTR may also function in Aβ clearance (Ribeiro et al. 2014). In a recent study, TTR was found to regulate LRP1 expression and thus its effects on Aβ clearance could be partially mediated via this mechanism (Alemi et al. 2016).

In APP23 mice, the APP overexpressing neurons produce elevated amounts of TTR in the presence of Aβ; this effect appears well before the appearance of neuropathological or behavioral signs of disease (Li et al. 2011). Some studies have detected a reduction in the TTR concentration in the CSF of AD and iNPH patients compared to non-demented controls and patients with other dementia (Merched et al. 1998, Gloeckner et al. 2008). To some extent, the lowered amount of TTR in CSF also seems to correlate with the severity of AD disease (Gloeckner et al. 2008).
3 Aims of the Study

The underlying pathogenesis of iNPH is unknown. By clarifying the molecular mechanisms of iNPH, it may be possible to develop more efficient diagnostics, to expand our understanding of the pathological cascades leading to Aβ deposition not only in iNPH but also potentially in AD, as well as providing a platform for the development of alternative treatment and therapeutic strategies to supplement the invasive shunt surgery available today.

The specific aims of the study were

I. To investigate the molecular mechanisms leading to the Aβ accumulation in iNPH and to study the potential similarities and differences in these mechanisms between iNPH and AD.

II. To elucidate the neuropathology further at the genetic level by analysing gene expression profiles in iNPH brain samples for alterations in the expression of genes linked to AD pathogenesis and to assess whether there is a correlation with observed Aβ pathology.

III. To examine the role of AD-associated polymorphisms in a polygenic manner in iNPH brain samples with respect to Aβ accumulation.
4 Materials and Methods

4.1 SUBJECTS

4.1.1 iNPH cohorts (Studies I, II and III)
The iNPH patient / sample cohorts were from the Kuopio NPH Register of Kuopio University Hospital (KUH) (www.uef.fi/nph). All patients in the register have been evaluated for presumed iNPH fulfilling the following criteria: 1) one to three symptoms typical of iNPH: impaired cognition, gait problems or urinary incontinence; 2) enlarged brain ventricles compared to the size of the sulci of cerebral convexities in CT or MRI (Leinonen et al. 2010). The biopsy procedure was performed by making a right frontal 12 mm burr hole approximately three cm laterally from the midline and close to the coronal suture of the skull under local anesthesia and sedation (ICP monitoring) or general anesthesia (shunt procedure). Prior to the insertion of an intraventricular catheter for 24-hour ICP monitoring or alternatively prior to insertion of the intraventricular shunt, one to three cylindrical cortical brain biopsies of two mm in diameter and three to seven mm in length were obtained with biopsy forceps or by standard 14G biopsy needle (Temno). Due to the natural limitations of clinical register, every patient listed does not have an identical set of documented parameters and thus the patient cohorts employed in different studies consist of different but overlapping series of patients. In study I, the samples were categorised as Aβ - (no visible Aβ plaques), Aβ + (less than hundred plaques per section) and Aβ ++ (more than hundred plaques per section). iNPH sample cohorts are presented in Table 5.

<table>
<thead>
<tr>
<th>Cohort and study</th>
<th>Brain Biopsy</th>
<th>N</th>
<th>Age at biopsy mean (years) ± SD</th>
<th>Female, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ- and β-secretase measurement cohort (Study I and III)</td>
<td>Aβ -</td>
<td>8</td>
<td>74.6 ± 5.9</td>
<td>3 (37.4)</td>
</tr>
<tr>
<td></td>
<td>Aβ +</td>
<td>18</td>
<td>77.4 ± 6.1</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Expression cohort iNPH (Study II)</td>
<td>Aβ-</td>
<td>10</td>
<td>75.2 ± 6.8</td>
<td>4 (40.0)</td>
</tr>
<tr>
<td></td>
<td>Aβ+</td>
<td>12</td>
<td>78.6 ± 6.8</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>CSF cohort (Study II)</td>
<td>Aβ-</td>
<td>43</td>
<td>71.8 ± 8.5</td>
<td>19 (44.2)</td>
</tr>
<tr>
<td></td>
<td>Aβ+</td>
<td>59</td>
<td>78.1 ± 5.0</td>
<td>36 (61.0)</td>
</tr>
<tr>
<td>SNP cohort (Study III)</td>
<td>Aβ-</td>
<td>104</td>
<td>77.8 ± 8.4</td>
<td>57 (54.8)</td>
</tr>
<tr>
<td></td>
<td>Aβ+</td>
<td>84</td>
<td>81.3 ± 5.5</td>
<td>51 (60.7)</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ- = no amyloid-β plaques, Aβ+ = amyloid-β plaques observed in biopsies, SD = standard deviation, iNPH = idiopathic normal pressure hydrocephalus, CSF = cerebrospinal fluid, SNP = single nucleotide polymorphism.
4.1.2 Neuropathological cohort (I)
Human post-mortem AD brain samples were obtained from Kuopio University Hospital (Kuopio Brain Bank, www.alzforum.org/brain-banks/kuopio-brain-bank). The set included inferior temporal lobe samples from 74 older individuals investigated within memory clinic research projects and later autopsied and evaluated for AD pathology (16 males and 58 females; mean age 83 ± SD 6.4 years). The CERAD criteria were used for the diagnosis of AD (Mirra et al. 1991). Patients were subdivided into three severity groups; mild (n = 35), moderate (n = 12) and severe (n = 27) according to Braak staging (1−2 = mild, 3−4 = moderate, and 5−6 = severe) (Braak et al. 2006).

4.1.3 Non-demented controls (II)
The controls for the expression data were frontal cortex samples from eight non-demented deceased individuals from the Kuopio Brain Bank (mean age 78.4 ± SD 13.8, range 46 to 89 years, 62.5 % female).

4.2 EVALUATION OF iNPH SYMPTOMS (II)
To classify a triad of disorders (impaired cognition, disturbed gait and urinary incontinence), we used the modified Finnish version of the 12-point idiopathic Normal Pressure Hydrocephalus Grading Scale (iNPHGS). iNPHGS is a clinician-rated scale to separately assess the severity of each of the three triad symptoms (Kubo et al. 2008). The scores are based on observations by the physician and interviews with the patient or their caregiver. Score ranges from 0 to 4 for each dimension, with higher scores representing worse symptoms. An alteration of one or more points was considered as a change. By comparing the iNPHGS score before the shunt procedure and one year after the shunt procedure, the patients were divided into two groups, “shunt response” and “no response”.

4.3 HISTOLOGY AND IMMUNOHISTOCHEMISTRY (I, II and III)
The iNPH biopsy samples were fixed in buffered formalin overnight and embedded in paraffin. The paraffin-embedded biopsy samples were sectioned (7 µm) and stained with hematoxylin-eosin, and immunostained with monoclonal antibodies directed to Aβ (6F/3D, M0872; Dako; dilution 1:100; pre-treatment 80 % formic acid 1 hour) and Pτ protein (AT8, 3Br-3; Innogenetics; dilution 1:30) (Leinonen et al. 2010). The Aβ burden was roughly quantified by counting plaques in the biopsy under a light microscope and dividing the total number of plaques by the area of the gray matter (mm²). Cellular or neuritic immunoreactivity for Pτ was evaluated by light microscopy in all samples and was graded as present or absent by a neuropathologist (Seppala et al. 2012). The Aβ burden was also quantified more precisely by a method described earlier (Seppala et al. 2012). Briefly, representative high-resolution images consisting of the cortical regions of interest were acquired at 2X magnification (Plan N2X/0.06) using an upright Olympus optical microscope (OLYMPUS BX40) with Olympus optical DP50 camera. A flatfield image was also acquired under similar settings to correct for uneven illumination. On the grey-scaled images, cortical regions of interest were outlined and selected using Lasso tools. Images were then thresholded to segregate plaques from the background. The number of pixels counted within selections, after calibration, gave corresponding areas in mm². The percentage of cortical area covered with stained antibody against Aβ was reported for the biopsy samples.
4.3.1 Soluble Aβ x-42 measurements (I)
Soluble Aβ x-42 (Aβ42) levels were measured from the soluble protein fraction of iNPH samples (see below). The soluble Aβ42 levels were determined using a monoclonal and HRP-conjugated antibody-based Human/Rat β Amyloid 42 (High Sensitive; 290 62601) ELISA Kit (Wako). After a 30-minute incubation at room temperature, the reaction was terminated and the absorbance was measured at 450 nm with an ELISA microplate reader (BioRad). Aβ42 concentrations were normalized to total protein concentration within each sample.

4.4 ENZYME ACTIVITY ASSAY

4.4.1 Processing of tissue samples (I and III)
Frozen samples from both iNPH and AD patients (study I) were mechanically homogenized in 400 µl of 1 x buffer B (20 mM Hepes pH 7.5, 150 mM KCl, 2 mM EGTA) with protease and phosphatase inhibitors (1:100; Thermo Scientific) in an ice bath. Protein aliquots were then ultracentrifuged (100000 x g, 50.4 Ti rotor; Beckman) for two hours at 4°C, and the supernatant (= soluble fraction) was collected for soluble Aβ x-42 peptide measurements. Subsequently, the remaining pellet was re-suspended in guanidine buffer (5 M guanidine-HCl / 50 mM Tris HCl, pH 8.0), incubated for 2 hours at room temperature on a shaker, and diluted 1:50 in BSAT-DPBS (5 % BSA/0.03 % Tween 20 in DPBS, pH 9.0) containing protease and phosphatase inhibitors. The suspension was centrifuged for 20 minutes at 15700 x g and the supernatant was collected for β- and γ-secretase enzyme activity assays.

4.4.2 β- and γ-secretase activity assays (I and III)
β-Secretase (Cat # K360-100, BioVision, CA, USA) activity was measured from the tissue homogenates according to the manufacturer’s instructions. Briefly, equal amounts of membrane protein fractions were incubated at 37 °C for 1 hour with the β-secretase-specific substrate peptides conjugated to fluorescent reporter molecules EDANS and DABCYL. Subsequently, the emitted light 510 nm was detected on a fluorescence microplate reader (Wallac) after EDANS excitation at 355 nm. The γ-secretase activity was measured from the tissue homogenates as previously described (Farmery et al. 2003). In brief, 60 µg (AD) / 20 µg (iNPH) of solubilized membrane protein preparation was incubated at 37 °C overnight in 150 µl of assay buffer containing 50 mM Tris-HCl, pH 6.8, 2 mM EDTA, 0.7 % CHAPSO (w/v), and 8 µM fluorogenic γ-secretase substrate (NMA-GGVVIATVK(DNP)-DRDRDR-NH 2, Cat # 565764, Calbiochem). After incubation, the samples were centrifuged at 15700 x g for 10 min and transferred to a 96-well plate. Fluorescence was measured using a plate reader (Fluorstar Galaxy) with an excitation wavelength of 355 nm and an emission wavelength of 440 nm. The background fluorescence from the substrate samples was subtracted in the final analysis. β- (β-Secretase Inhibitor III, GL189, Cat # 565780, Calbiochem; 150 µM/ reaction) and γ- (L-685,458; Cat # L1790, Sigma-Aldrich; 100 µM/ reaction) secretase inhibitors were used in a subset of samples to validate the specificity of the β- and γ-secretase activities.
4.5 CSF SAMPLES AND BIOMARKER ANALYSES (II)

The ventricular CSF samples (TTR, n = 69; sAPP, n = 68) 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 91 (TTR) and 94 (sAPP) patients. The lumbar samples were obtained after a lumbar puncture prior to the ICP measurement protocol or shunting. sAPP isoforms (sAPPα, sAPPβ) were analyzed using commercially available multiplexed assays (Meso Scale Discovery, Gaithersburg, MD, USA) (Jeppsson et al. 2013, Zetterberg et al. 2008). TTR was analyzed using a nephelometric method (Immage® Immunochemistry System, Beckman Coulter, Brea, CA). 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.

4.6 MICROARRAY-BASED GENE EXPRESSION AND SPLICING ANALYSIS (II)

The expression microarray was performed at the Finnish Microarray and Sequencing Centre in Turku, Finland as described previously (Martiskainen et al. 2015b). The chips were scanned by the Agilent Technologies Scanner, model G2565CA. The numerical data was acquired with the Agilent Feature Extraction program version 10.7.3. There was more than one probe targeted to TTR, APP and ADAM10 and the results attained with the different probes targeting to the same sequence at the 3’ end of mRNA were similar.

4.7 POLYGENIC RISK ANALYSIS (III)

4.7.1 DNA extraction and genotyping

DNA was extracted from peripheral blood. Single nucleotide polymorphisms (SNPs) were genotyped using Sequenom iPlex platform (Sequenom, Hamburg, Germany) at the University of Eastern Finland. Samples with an average call rate of 90 % were included. SNPs for the association analysis were in Hardy-Weinberg equilibrium. The SNPs genotyped are presented in table 6.

4.7.2 Polygenic risk score calculations

In order to study the combined effect of the SNPs associated with AD risk, a polygenic risk score was calculated based on 22 GWAS-identified AD risk loci (the 12 loci based on findings in three meta-analysis papers (Lambert et al. 2009, Lambert et al. 2013a, Lambert et al. 2013b). and AlzGene Top10 loci) as described previously (Martiskainen et al. 2015a). Briefly, the risk score for each individual was calculated by summing log-transformed odds ratios reported in the meta-analyses (Bertram et al. 2007, Lambert et al. 2013b), weighted by the number of alternative alleles carried by the individual. Since APOE4 is known to have the strongest risk effect and is therefore likely to manipulate the polygenic risk score, we also calculated a polygenic risk score excluding APOE4 as well as a risk score based on APOE4 genotype alone.
Table 6. SNPs associated with AD risk and genotype frequencies.

<table>
<thead>
<tr>
<th>Chr, gene</th>
<th>SNP</th>
<th>Genotypes</th>
<th>Genotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CR1</td>
<td>rs3818361</td>
<td>CC/CT/TT</td>
<td>0.67/0.28/0.05</td>
</tr>
<tr>
<td>2. BIN1</td>
<td>rs744373</td>
<td>AA/AG/GG</td>
<td>0.58/0.32/0.10</td>
</tr>
<tr>
<td>2. INPP5D</td>
<td>rs35349669</td>
<td>CC/CT/TT</td>
<td>0.65/0.28/0.07</td>
</tr>
<tr>
<td>6. CD2AP</td>
<td>rs9349407</td>
<td>GG/GC/CC</td>
<td>0.69/0.28/0.03</td>
</tr>
<tr>
<td>6. HLA_DRB5</td>
<td>rs9271192</td>
<td>AA/AC/CC</td>
<td>0.59/0.35/0.06</td>
</tr>
<tr>
<td>7. EPHA1</td>
<td>rs11771145</td>
<td>GG/AA/AG</td>
<td>0.34/0.20/0.46</td>
</tr>
<tr>
<td>7. NME8</td>
<td>rs2718058</td>
<td>AA/AG/GG</td>
<td>0.46/0.40/0.14</td>
</tr>
<tr>
<td>8. CLU</td>
<td>rs11136000</td>
<td>CC/CT/TT</td>
<td>0.37/0.41/0.22</td>
</tr>
<tr>
<td>8. PTK2B</td>
<td>rs28834970</td>
<td>TT/CC/CT</td>
<td>0.49/0.12/0.39</td>
</tr>
<tr>
<td>10. FRMD4A</td>
<td>rs7081208</td>
<td>GG/GA/AA</td>
<td>0.63/0.35/0.02</td>
</tr>
<tr>
<td>10. FRMD4A</td>
<td>rs2446581</td>
<td>GG/GA/AA</td>
<td>0.74/0.23/0.03</td>
</tr>
<tr>
<td>10. FRMD4A</td>
<td>rs17314229</td>
<td>CC/CT/TT</td>
<td>0.83/0.17/0</td>
</tr>
<tr>
<td>11. CELF1</td>
<td>rs10838725</td>
<td>TT/CC/CT</td>
<td>0.59/0.11/0.30</td>
</tr>
<tr>
<td>11. MS4A6A</td>
<td>rs610932</td>
<td>GG/GT/TT</td>
<td>0.42/0.48/0.10</td>
</tr>
<tr>
<td>11. MS4A4E</td>
<td>rs670139</td>
<td>GG/GT/TT</td>
<td>0.17/0.47/0.36</td>
</tr>
<tr>
<td>11. PICALM</td>
<td>rs3851179</td>
<td>GG/GA/AA</td>
<td>0.48/0.40/0.12</td>
</tr>
<tr>
<td>11. SORL1</td>
<td>rs11218343</td>
<td>TT/CC/CT</td>
<td>0.80/0.02/0.18</td>
</tr>
<tr>
<td>14. FERMT2</td>
<td>rs17125944</td>
<td>TT/CC/CT</td>
<td>0.80/0.02/0.18</td>
</tr>
<tr>
<td>14. SLC24A4</td>
<td>rs10498633</td>
<td>GG/GT/TT</td>
<td>0.72/0.25/0.03</td>
</tr>
<tr>
<td>18. DSG2</td>
<td>rs8093731</td>
<td>CC/CT/TT</td>
<td>0.79/0.18/0.03</td>
</tr>
<tr>
<td>19. ABCA7</td>
<td>rs3764650</td>
<td>TT/TG/GG</td>
<td>0.95/0.05/0</td>
</tr>
<tr>
<td>19. APOE</td>
<td>ε4 0/1/2</td>
<td></td>
<td>0.40/0.50/0.10</td>
</tr>
<tr>
<td>19. CD33</td>
<td>rs3865444</td>
<td>CC/CA/AA</td>
<td>0.57/0.30/0.13</td>
</tr>
<tr>
<td>20. CASS4</td>
<td>rs7274581</td>
<td>TT/CC/CT</td>
<td>0.83/0.02/0.15</td>
</tr>
</tbody>
</table>

Abbreviations: AD = Alzheimer’s disease, Chr = chromosome, SNP = single nucleotide polymorphism
4.8 STATISTICAL ANALYSES

4.8.1 Study I
Statistical analyses were performed using the SPSS program (version 19.0). One-way ANOVA with a post-hoc test (LSD) was used in the statistical analyses of biochemical data. Comparisons between groups were made using independent samples t-test. Correlations were determined using Pearson’s correlation coefficient. Values are indicated as mean ± SE. The level of statistical significance was set to p < 0.05.

4.8.2 Study II
Statistical analyses on the CSF data were performed using the SPSS software (version 19.0). Comparisons between groups were made using independent samples t-test. Correlations were determined using Pearson’s correlation coefficient. The level of statistical significance was set at p < 0.05. Microarray analyses were performed with R statistical software version 3.0.1 (R Development Core Team, 2009) and Bioconductor version 2.13 (Gentleman et al. 2004). Data import was done with the limma package version 3.16.7 (Llimma 2005). The probe signal intensities were background-corrected using “normexp” method in the limma package. Quantile normalization was used to bring the array signal intensities to comparable levels against each other. Average expression levels between duplicate probes were used as the signal for the probe. In the gene expression analysis, 3’UTR (untranslated region) probe signal was used as a measure of the respective gene’s global expression level. If multiple such probes targeted the same gene, the average value of the probes was used. All statistical analyses were performed using the log2 expression values. The 3’UTR probe signals were used for correlation analyses using Pearson’s correlation and corrected for multiple testing using Bonferroni correction. To investigate differential expression in Aβ -related pathways globally, we searched for all Aβ -related pathways from MSigDB database v5.0 (Subramanian et al. 2005). Four such pathways were identified (REACTOME_AMYLOIDS, AMYLOID_PRECURSOR_PROTEIN_METABOLIC_PROCESS, KEGG_ALZHEIMERS_DISEASE, BIOCARAT_PLATELETAPP_PATHWAY). Additionally, we created a gene set from the ALZGENE database based on our previous study (I) (APP, ADAM9, ADAM10, ADAM17, BACE1, PSEN1, PSEN2, PEN2, NCT, APH1, TTR). We used GSEA 2.2.0 (Subramanian et al. 2005) to analyze the enrichment of genes in the top or bottom of the gene list ranked by signal to noise ratio and using weight p = 1. To evaluate statistical significance, we permuted case/control label for 10000 times and counted how many times we observed a pathway score higher than in the actual dataset. Consequently, REACTOME_AMYLOIDS pathway was found to be significantly involved after GSEA-based multiple testing correction. The Aβ pathway genes of Reactome were visualized by complete linkage hierarchical clustering using gplots R-package. Selected pathway gene correlation p-values were multiplied by the number of genes. The p-values < 0.05 were considered significant.

4.8.3 Study III
Statistical analyses were performed using R (version 3.2.2). Individual SNPs were analysed using additive genetic model. Continuous outcome variables were analysed with linear regression, and categorical variables were analysed using logistic regression. The analyses were performed using a model with only the individual SNP or genetic risk score as an independent variable, and additionally with models adjusted for 1) age, and 2) age and sex. To account for multiple testing, we used Benjamini-Hocberg false discovery (P-FDR) to adjust the p-values and considered P-FDR < 0.05 to be statistically significant. The comparison between main and sub-cohort was done by employing student’s t-test with SPSS software, version 21.0.
4.9 ETHICS STATEMENT
This study was approved by the Kuopio University Hospital (KUH) Research Ethical Committee. All participants or their trustees gave an informed, written consent prior to participating in the study. In the case when a clinician considered that dementia may significantly affect the competence of the patient to give consent, a next of kin, caretaker or guardian gave the consent on behalf of the participant. When the consent was given by a participant’s trustee, the patient’s own opinion was consulted and no patients were recruited against their will.
5 Results

5.1 γ-SECRETASE, BUT NOT BACE1 ACTIVITY, IS INCREASED IN THE FRONTAL CORTEX OF iNPH PATIENTS WITH AD-LIKE Aβ PATHOLOGY

We measured the activity of γ-secretase and BACE1 in iNPH (n = 26) and AD (n = 74) samples. Both secretase activity assays were validated using specific BACE1 and γ-secretase inhibitors. The secretase activity was compared to the amount of Aβ plaques in iNPH samples and the disease severity according to Braak staging in AD samples (Braak et al. 2006). The amount of Aβ plaques was based on visual evaluation by neuropathologists (Seppala et al. 2012); this procedure has been shown to correlate with results obtained by a more specific quantitation of plaques and with the amount of soluble Aβ42 measured from the samples. The samples were categorised as Aβ - (no visible Aβ plaques), Aβ + (less than hundred plaques per section) and Aβ ++ (more than hundred plaques per section). Patients with AD were subdivided in three severity groups; mild (n = 35), moderate (n = 12) and severe (n = 27) according to Braak staging (1−2 = mild, 3−4 = moderate, 5−6 = severe) (Braak et al. 2006). In the AD samples, soluble Aβ42 levels were increased significantly in relation to disease severity, while the soluble Aβ42 levels were robustly lower in the Braak 0 group as compared to mild, moderate and severe groups. It was also verified that the activity of BACE1 or γ-secretase did not significantly correlate in the AD cohorts with the post-mortem delay (r = −0.177, p = 0.10 and r = −0.101, p = 0.36, respectively) or with the age of death (r = 0.142, p = 0.20 and r = 0.096, p = 0.38, respectively). Furthermore, the activity of BACE1 or γ-secretase did not significantly correlate with the age of iNPH patients at the biopsy (r = −0.183 and p = 0.59 and r = −0.110 and p = 0.40, respectively).

5.1.1 γ-secretase and BACE1 activity in iNPH and AD brain samples

In the iNPH specimens, the relative γ-secretase activity was significantly higher in samples with Aβ plaques (Aβ + and Aβ ++ samples) as compared to the iNPH samples without any detectable Aβ plaques (p = 0.009; figure 3A and 3B). On the contrary, when the relative γ-secretase activity of the inferior temporal cortex samples of AD patients was assessed, no significant differences were observed with regard to disease severity (figure 3C).

The opposite was seen with BACE1 activity, which was not significantly altered between the different Aβ groups of the iNPH samples (figure 4A and 4B), whereas a significant difference was observed with regard to AD severity, (p = 0.0003 between mild and severe and p = 0.02 between mild and moderate; figure 4C). BACE1 activity was also significantly increased in the severe group when they were compared to the Braak 0 group.

However, the activity of BACE1 or γ-secretase did not significantly correlate with the percentual Aβ staining values (r = −0.200, p = 0.47 and r = −0.101, p = 0.69, respectively). In iNPH samples, BACE1 and γ-secretase activity displayed non-significant tendency to correlation (r = 0.399, p = 0.06), while a clear correlation between the activity of these two secretases was found in the AD sample set (r = 0.556, p = 0.0000003).
Figure 3. γ-secretase activity is increased in the frontal cortex of iNPH patients with Aβ pathology but not in AD samples with regard to disease severity. A. Significant increase in γ-secretase activity is observed Aβ +/++ samples compared to Aβ - samples. Aβ - = no plaques, Aβ + = less than 100 plaques and Aβ ++ = more than 100 plaques per section, **p = 0.009 in iNPH samples. B. Increase in γ-secretase activity (not significant) can be observed along with increased Aβ status in iNPH samples, *p = 0.08. C. Significant increase is not seen in γ-secretase activity in the inferior temporal cortex of AD patients in relation to disease severity according to Braak staging (1-2 = mild, 3-4 = moderate, and 5-6 = severe). Data is shown with the standard error of the mean.
Figure 4. BACE1 activity is unaltered in the frontal cortex of iNPH patients with Aβ pathology but is significantly increased in AD samples in relation to disease pathology. Visual plaque count from immunostained iNPH brain sections. Aβ - = no plaques, Aβ + = less than 100 plaques and Aβ ++ = more than 100 plaques per section. A, B. Between different iNPH sample groups no significant alteration in BACE1 activity is observed. C. BACE1 activity in AD is significantly increased in the inferior temporal cortex of AD patients with disease severity (Braak staging; 1−2 = mild, 3−4 = moderate, and 5−6 = severe, \( p^* = 0.02, p^{**} = 0.0003 \)). Data are shown with the standard error of the mean.
5.2 THE EXPRESSION OF APP IS INCREASED AND THE EXPRESSION OF TRANSTHYRETIN IS DECREASED IN iNPH (II)

By utilizing a microarray chip approach, the genome wide expression profile of ~35 000 probes were delineated in 22 iNPH patients and eight non-demented control subjects (Agilent 8x60K chip containing custom-designed probes and default probes for specific AD-related genes). The basic concept was to study iNPH-related expression changes in frontal cortex biopsy samples. The results were corrected for multiple testing.

In the expression of TTR, APP and ADAM10, a statistically significant difference was seen between iNPH and control samples (figure 5). In comparison to control samples, on average the mRNA levels of TTR were 17-fold lower in iNPH samples. In contrast, the mRNA levels of APP were on average three times higher in iNPH samples in comparison to control samples. Furthermore, from the selected set of pathway genes related to APP (BACE1, ADAM9, ADAM10, ADAM17 and γ-secretase subunits), the expression of ADAM10 in iNPH patient samples was increased as compared to control samples. No statistically significant correlation was found between the expression of these genes and Aβ or HPτ pathology of the frontal cortex. In addition, a similar expression profile of the three APP isoforms (APP695, APP751, and APP750) was seen in iNPH patients with or without Aβ pathology (Aβ+ versus Aβ–). The severity of symptoms or the shunt response did not correlate with the expression of ADAM10, APP or TTR. Furthermore, the expression of these genes did not correlate with the post-mortem delay. On the contrary, a weak correlation between age and APP expression was seen in the iNPH samples (r = 0.43, p = 0.04). No differences in the expression of other genes important for the production of Aβ (as mentioned earlier, BACE1, ADAM9, ADAM17 and components of γ-secretase) were observed between the iNPH and control group.

![Figure 5](image_url). The expression levels of APP, TTR and ADAM10 differ significantly between iNPH patient samples and non-demented controls. The expression levels in iNPH samples (n = 22) were normalized to non-demented control samples (ND, n = 8). ***p = 1.8 x 10⁻⁹ – 2.0 x 10⁻¹⁴, *p < 0.05; mean ± SD.
5.3 THE CSF CONCENTRATIONS OF SOLUBLE APP ISOFORMS AND TTR IN iNPH PATIENTS (II)

The levels of sAPPα, sAPPβ, and soluble TTR were measured from both the ventricular and lumbar CSF samples of iNPH patients. In the ventricular CSF samples, sAPPα levels positively correlated with levels of sTTR ($r = 0.24$, $p = 0.05$). In the lumbar samples, the levels of sAPPα positively correlated with those of sAPPβ. The levels of both sAPPα and sAPPβ displayed a significant positive correlation with the levels of sTTR (table 7). The absolute levels of sAPP species were significantly lower in the ventricular samples as compared to the lumbar samples ($p < 0.0001$). The presence or absence of Aβ plaques or the HPτ pathology determined from the frontal cortex did not correlate with the protein levels of sAPPα, sAPPβ, and sTTR in the CSF, except that lumbar sAPPβ did display a weak correlation to HPτ ($p = 0.04$, $r = 0.22$). In addition, the levels of sTTR or sAPPα/β did not differ between groups determined by the shunt response. Furthermore, the correlation of gender, age, and pre-operative MMSE score with the ventricular and lumbar levels of CSF proteins were analyzed. Female iNPH patients presented with significantly higher levels of ventricular sAPPα ($p < 0.05$), sAPPβ ($p = 0.04$), and sTTR ($p = 0.02$) in comparison to male iNPH patients. No differences were seen in lumbar CSF protein levels.

**Table 7.** Correlation analysis between the levels of sAPPα, sAPPβ and TTR in the lumbar CSF of iNPH patients.

<table>
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<th>sAPPα lumbar CSF</th>
<th>sAPPβ lumbar CSF</th>
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<td>sAPPα</td>
<td>1</td>
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<td>lumbar CSF</td>
<td>Pearson’s Correlation</td>
<td>p-value</td>
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<td>p-value</td>
<td>&lt;0.0001</td>
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<td>N</td>
<td>94</td>
<td>94</td>
<td>91</td>
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<tr>
<td>sAPPβ</td>
<td>0.96</td>
<td>1</td>
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<tr>
<td>lumbar CSF</td>
<td>Pearson’s Correlation</td>
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<td>n</td>
<td>94</td>
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Abbreviations: sAPPα = soluble APPα; sAPPβ = soluble APPβ; CSF = cerebrospinal fluid; TTR = transthyretin.
5.4 Aβ PATHOLOGY SEEN IN iNPH IS ASSOCIATED WITH APOE4 (III)

The sample set used in this study consisted of 188 brain samples from suspected iNPH patients. The samples were divided into two groups according to the presence or absence of Aβ pathology. The polygenic risk score was calculated based on 22 GWAS-identified AD risk loci (the 12 loci based on findings in three meta-analysis papers (Lambert et al. 2009, Lambert et al. 2013a, Lambert et al. 2013b) and AlzGene Top10 loci) as described previously (Martiskainen et al. 2015a). First we compared separately the non- APOE4 polygenic risk score and the plain APOE -status to the Aβ pathology in the brain samples. After adjusting for age and gender only, APOE4 displayed a significant association to Aβ status ($r^2 = 0.353$, $p = 0.0006$). The APOE4 burden also had a significant association with the percentual Aβ burden in the samples ($r^2 = 0.530$, $p = 1.6 \times 10^{-6}$). The APOE ε4 allele was in fact encountered more frequently in Aβ+ samples (45.2 % APOE4 carriers) than in Aβ samples (13.5 % APOE4 carriers). In addition, in a sample subset (n = 11), γ-secretase activity was linked to SNP rs9349407 (CD2AP) ($p = 0.016$). We also compared the polygenic risk score to β-secretase activity and to the MMSE score of the patients before the shunt surgery, but no significant association was detected.
6 Discussion

6.1 PRODUCTION OF Aβ

The non-pathologic role of Aβ is poorly understood, though it has been hypothesized to be crucial for memory (Ohno 2016) or to possess anti-microbial qualities (Kumar et al. 2016) when present in physiological levels. In AD, the accumulation of Aβ and HPτ is considered as a hallmark feature (Braak and Braak 1991, Thal et al. 2002). Aβ plaques are frequently found also in iNPH (Leinonen et al. 2012a, Leinonen et al. 2012b), but the role and formation of Aβ plaques in iNPH are still to be clarified. The typical symptoms of iNPH have been suggested to be due to physical stress caused by enlarged ventricles, resulting in ischemia in brain tissue (Fisher 1982, Conner et al. 1984, Ohata and Marmarou 1992, Waldemar et al. 1993, Uhl et al. 1999). The CSF turnover is also considered to be disturbed, leading to the impairment in the clearance of the toxic metabolites from brain tissue and further to accumulation of Aβ (Silverberg et al. 2003). Shunting is based on the hypothesis that when the intracranial pressure is repaired, also the CSF circulation recovers (Jeppsson et al. 2013). In fact, in a recent study, the CSF levels of Aβ42 have been shown to increase after shunting (Moriya et al. 2015). However in rats with induced hydrocephalus, no significant difference was seen in the amount of Aβ plaques between elderly hydrocephalic rats and age-matched controls (Deren et al. 2009). However, it does not exclude the possibility that the pathological mechanisms of ventriculomegaly in true iNPH could share at least some common steps with Aβ accumulation. Whether the accumulation of Aβ is dependent on the CSF circulation remains unclear. It is also unknown why Aβ plaques do not appear invariably in every iNPH patient.

In outline, the formation of plaques can be due to an increase in the production, alteration in the transport and storage, defects in clearance, or a combination of defects in these processes. There are some results suggesting that in AD the impaired clearance would be most crucial for Aβ accumulation (Mawuenyega et al. 2010). It is most likely that the accumulation results from an imbalance between production and clearance.

6.1.1 The alterations in APP and ADAM10 expression

The global expression analysis performed on iNPH samples showed that the expression of APP had been dramatically increased when compared to non-demented control samples (study II). In previous studies, no alterations in the level of APP have been detected in LOAD (Gatz et al. 2006, Medway and Morgan 2014). Thus, our study suggests for the first time that events in Aβ production differ between these two conditions. In LOAD, the total expression levels of APP remain unaltered but there are some qualitative changes favouring the expression of KPI domain isoforms (Preece et al. 2004, Matsu et al. 2007, Martiskainen et al. 2015b). In iNPH patients, no such alterations were observed in relative expression levels of different APP isoforms (study II). Thus it would seem that in iNPH, the total amount of APP could be crucial in Aβ generation. In our study, the APP expression weakly correlated with age, even though it has been shown previously that Aβ plaque formation is, to some extent, age dependent and occurs also in cognitively healthy individuals (Pike et al. 2007, Jack et al. 2009). Moreover, also the expression of ADAM10 was significantly increased in iNPH, which might be a compensatory mechanism against the enhanced production of APP (study II). ADAM10 is considered as the main α-secretase in the non-amyloidogenic cascade and thus a shift towards less harmful processing of APP might be a potential neuroprotective event in iNPH. In fact, it has been shown in a study examining two lines of knock-in mice with replaced endogenous APP locus that the formation of soluble product of APP cleavage by α-secretase (sAPPα) is alone somewhat
neuroprotective against the pathologic Aβ accumulation (Ring et al. 2007). However, increased expression of ADAM10 does not automatically translate into increased α-secretase activity.

6.1.2 The BACE1 and γ-secretase

We found that iNPH differs from AD in terms of the activity profiles of two secretases crucial for amyloidogenic cascade (study I). Those iNPH samples exhibiting Aβ plaques showed enhanced γ-secretase activity as compared to samples without Aβ plaques (study I). In our study, the γ-secretase activity was also increased in relation to the increasing amount of plaques. This could support the hypothetical correlation between Aβ production and γ-secretase activity. The importance of these results was highlighted by the finding that in AD samples, no quantitative alteration in γ-secretase activity was seen in regard to disease severity (study I). In fact, there was no significant augmentation in γ-secretase activity in AD compared to non-demented control samples in a previous study (Kakuda et al. 2012). There is some evidence that γ-secretase activity could be up-regulated under oxidative stress and hypoxic conditions (Pluta 2007, de la Torre 2008, Li et al. 2009b), and in iNPH, these conditions could exist in the brain tissue displaced by the enlarged ventricles. However, hypoxia is also considered to occur in AD brain but potential ensuing up-regulation in γ-secretase activity seems to be absent. Instead some qualitative alterations in γ-secretase activity have been described in AD literature and these changes could potentially favour the production of the Aβ42 peptide (Kakuda et al. 2012). It has been speculated that the shunting procedure would lead to changes in γ-secretase activity (Kakuda et al. 2013, Moriya et al. 2015).

In contrast to γ-secretase activity, BACE1 activity did not differ in iNPH samples in regard to Aβ status (study I). In our AD cohort, the BACE1 activity increased with disease severity. This is in line with previous findings, i.e. AD patients display approximately 30 – 50 % increase in the BACE1 activity compared to age-matched, non-demented subjects (Fukumoto et al. 2002, Yang et al. 2003, Ahmed et al. 2010). Increased BACE1 activity links to a higher production rate of Aβ40 and Aβ42 both in vitro and in vivo (Sun et al. 2006). According to results from mouse experiments, it has been hypothesized that inhibition of BACE1 could be well tolerated and thus offer a potential therapeutic target for AD (Ohno 2016). Similar to the situation with γ-secretase, hypoxia and ischemia induce BACE1 levels and activity (Wen et al. 2004, Zhang et al. 2007).

It is possible that in both AD and iNPH, ischemic stress is a very important event in Aβ pathology, and thus it is somewhat puzzling that the pattern of secretase activities in Aβ cascade seems to differ in these conditions. It is likely that in addition to hypoxia, more complex metabolic disturbances taking place over time are required. This idea is supported by the fact that no increase in Aβ accumulation is seen for example after an ischemic stroke (Sahathevan et al. 2016). One potential explanation is that on average AD is a more drastic condition than iNPH and thus the metabolic disturbances in AD brain might well be more profound. Even though these two conditions result in similar plaque deposition, it is far from certain that they share any common pathological processes. The accumulation of Aβ might be purely analogical.
6.1.3 Soluble products of APP processing

According to our results, lumbar levels of sAPPβ showed only a weak correlation with HPτ (study II). The significance of this finding in regard to iNPH is difficult to evaluate, since HPτ in general is not specific for the condition. Both sAPP species have also been shown earlier to present in higher absolute levels in lumbar than ventricular CSF (Ray et al. 2011, Miyajima et al. 2013, Jeppsson et al. 2013). In addition, in our study, female patients displayed higher amounts of studied proteins in ventricular CSF than males. Both of these findings are logical in terms of CSF dynamics and anatomic traits.

The soluble by-products of APP processing (sAPPα and sAPPβ) have been considered as potential biomarkers (Serot et al. 1997, Castano et al. 2006, Hansson et al. 2009). It has been shown that sAPPα and sAPPβ are lower in the CSF of iNPH patients in comparison to healthy individuals and AD patients (Ray et al. 2011, Jeppsson et al. 2013, Miyajima et al. 2013). In comparison, a meta-analysis performed on AD noted that CSF levels of neither sAPPα nor sAPPβ displayed any significant difference between AD patients and controls (Olsson et al. 2016). On the other hand, the CSF Aβ42 concentration is typically decreased in AD, which also occurs in iNPH, and negatively correlates to brain Aβ deposition (Motter et al. 1995, McKhann et al. 2011, Pykkko et al. 2014). In addition, in AD, the level of HPτ was elevated in CSF, which in turn is not encountered in iNPH (Blennow et al. 1995, Seppala et al. 2012). One explanation is that the sAPP species remain in the brain tissue of iNPH patients more persistently than in AD due to their decreased turnover rate of CSF (Silverberg et al. 2003, Graff-Radford 2014). The impaired CSF dynamics could lead to insufficient clearance of proteins from the brain tissue, which together with decreased levels of TTR might contribute to plaque formation. Alternatively, since sAPP peptides can be considered as markers of the Aβ cascade, this difference could potentially serve as an additional proof that there are differences in APP processing between iNPH and AD. In line with the previous findings (Pykkko et al. 2014), the CSF sAPP species did not correlate with brain Aβ pathology or to the response to shunt treatment (II).

6.2 TTR AND ApoE4

In this work, two proteins potentially linked to Aβ cascade were examined, TTR and ApoE4. The expression of TTR was decreased in iNPH compared to non-demented controls (study II) and APOE4 allele correlated with Aβ pathology (study III). In AD, the effects of these proteins on disease progression are considered opposite to one another. APOE4 is a well-known risk gene for both EOAD and LOAD, and TTR is thought to possess protective functions.

In AD, the level of TTR expression is known to increase and TTR itself is considered to confer protection against the harmful effects of Aβ (Buxbaum et al. 2008, Hakim and Adams 1965, Liu and Murphy 2006, Costa et al. 2008b, Costa et al. 2008a). This has not been shown in iNPH. We showed that in iNPH, the CSF sTTR levels did not correlate with disease severity (study II) as is known to occur in AD (Gloeckner et al. 2008), or with the shunt response or brain Aβ pathology (study II). In both iNPH and AD, the CSF sTTR levels are decreased when compared to other dementing conditions and non-demented controls (Serot et al. 1997, Merched et al. 1998, Gloeckner et al. 2008, Hansson et al. 2009, Schultz et al. 2010). The decrease in the TTR expression would nevertheless point to a role of TTR in iNPH pathogenesis, even although it seems that the importance of TTR as a neuroprotective mediator is not the same in iNPH as in AD (study II). It is also quite possible that the neuroprotective system in AD manifested as the elevated TTR activity fails in iNPH, and this promotes the formation of the plaques. In a very recent study, TTR has been shown to interact with C99 fragment released by BACE1 cleavage in the amyloidogenic cascade and thus it hinders the formation of Aβ (Li et al. 2016). This provides a potential link between the increased production of TTR and BACE1 activity seen in AD. If the increased BACE1 activity contributes to the enhanced production of TTR, it
would also explain why TTR expression is not augmented in iNPH. It is also possible that APP and TTR could be linked at the level of expression. We found no correlation in the expression levels, but this does not exclude that there might be a common mediator; naturally this is somewhat hypothetical. Instead, it has been shown that the sAPP species (sAPPα/β) could mediate the expression of TTR, linking it to both APP and BACE1 via Aβ cascade (Stein and Johnson 2002, Li et al. 2010). In fact, we noted that in iNPH, the lumbar sTTR levels correlated with both sAPP isoforms, and the correlation between ventricular sTTR and sAPPα was also seen (study II). Nonetheless, it remains unclear why the expression of TTR is not increased if there is an abundance of APP possibly followed by enhanced APP processing. If sAPP species mediate the TTR expression, they might also have a limiting function at such high concentrations.

In our polygenic risk score analysis, only APOE4 displayed a correlation with Aβ deposition in the brains of iNPH patients (study III). A similar correlation between APOE4 and Aβ accumulation has been shown also in non-demented individuals and patients with amnestic MCI and in AD (Seppala et al. 2012, Bangen et al. 2016). APOE4 is also associated with decreased CSF levels of Aβ42 in AD (Galasko et al. 1998, Martiskainen et al. 2015a).

In iNPH, an earlier study revealed that APOE4 is an independent predictive factor for Aβ accumulation, but it does not predict development of iNPH (Pyykkö et al. 2012). On the contrary, in AD the APOE4 allele is a major risk factor (Corder et al. 1993, Bu 2009, Huang and Mucke 2012), associated with early onset age and a more rapid disease progression (Roses 1996). ApoE4 is considered to affect primarily the clearance of Aβ (Castellano et al. 2011, Selkoe and Hardy 2016). It would seem that although the role of ApoE4 in disturbed Aβ metabolism might be similar in iNPH and AD, the other functions affecting disease pathology might be completely different. In AD, ApoE4 is potentially of greater significance for the disease progression, affecting various other processes in addition to Aβ accumulation. The meaning of ApoE4 for lipid transport, neuronal signaling, glucose metabolism, mitochondrial functions and neuroinflammation are acknowledged (Chen et al. 2010, Liu et al. 2013a). Alterations in these processes might be more dramatic in AD than in iNPH. Nevertheless, APOE4 is only a risk factor for AD, and not all AD patients carry the ε4 allele.

Of the other potential risk loci studied, in AD ABCA7 (rs3764650) and CD2AP (rs9349407) have been shown to associate with the amyloid burden (Shulman et al. 2013). Neither of these correlated with Aβ burden in iNPH, although in a small subcohort, CD2AP loci displayed a weak correlation with the γ-secretase activity (study III). However in our study, the subcohort was very small (n = 11). In AD, a similar polygenic risk score did display correlation with the γ-secretase activity (Martiskainen et al. 2015a). Despite the small cohort size, the fact that in AD these loci do correlate with Aβ deposition but not in iNPH, is interesting and emphasises the potential difference in mechanisms behind the development of Aβ plaques.

6.3 STRENGTHS AND LIMITATIONS OF THE STUDY

The greatest value of this work is in its pioneering settings so that the results can serve as a template for future research. These kinds of studies performed on biopsy and CSF samples collected from living patients are very rare; they offer an interesting view into the molecular processes taking place during the disease progression. In addition, the patient cohort employed is in fact rather large in comparison to many previous studies. iNPH has not been studied extensively, especially its molecular pathology is poorly known. The accumulation of Aβ in iNPH and AD has been considered as a similar process but according to the results of this work, it would seem that the events leading up to plaque formation might be very different. The comorbidity of iNPH and AD is frequent and hence they might share some common triggers or exposing factors but they would still represent two separate conditions.
A common problem with clinical cohorts is the variability of parameters available for each patient. Furthermore although it is advantageous to work with brain biopsies, the amount of material is very small and it is mandatory to prioritize which analyses should be performed. Good quality, non-demented brain tissue is not readily obtained for control purposes, which remains a constant deficiency in all our study settings.

In the comparison of the results obtained from AD and iNPH samples (study I), there are some potentially confounding factors. First, the iNPH and AD samples represented different brain regions. This could explain the differences seen in BACE1 and γ-secretase activities. However, the reason for the selection of different brain regions was the fact that these regions are known to be strongly affected in each condition. In iNPH, the frontal cortex and in AD the temporal cortex show the strongest Aβ pathology. Even if the AD samples would have included the frontal cortex, the disease driven alterations are less extensive in this region and thus most likely no increased γ-secretase activity would have been seen.

In the secretase analysis, the measurement of α-secretase activity in iNPH would have been highly informative. It would have been interesting to examine whether the increase in ADAM10 expression would actually translate into the increase in α-secretase activity as well. This would have provided proof for an interesting hypothesis i.e. the increase in ADAM10 expression manifests as a compensation towards increased APP production. Unfortunately the amount of brain material was very limited and no additional activity analysis could be performed.

Post-mortem delay is also a factor to consider, affecting both AD samples and non-demented control samples (study II). In both occasions, the post-mortem delay did not show any correlation to the parameters measured and thus the observed differences are not likely due to the use of control samples collected on autopsy. In case of both AD and non-demented controls, the acquirement of tissue from live patients is very difficult, since brain surgeries are not performed solely based on tissue sampling. In every study, the age and sex of the patients / subjects were taken into account and their potential effects on the results were contemplated.

An unfortunate limitation is the lack of CSF from the non-demented controls (study II). Although we did compare the results to values in the literature, the analysis of control samples would have provided additional reliability for results and allowed more rigorous discussion.

Perhaps the most important aspect requiring reflection is the difficulty of performing reliable diagnostics. As discussed in section 2.5.5, differential diagnostics of iNPH can be quite challenging and iNPH can be misdiagnosed as other neurodegenerative condition or cerebrovascular disease (Leinonen et al. 2010, Magdalinou et al. 2013, Jingami et al. 2015). Also the frequent presence of comorbid diseases can lead to diagnostic challenges and also affect the results obtained in the studies. iNPH itself is a very non-uniform condition and it is likely that with growing knowledge, the definition and nature of iNPH as a single disease will be challenged. Even though we cannot say with complete certainty that all the patients are "pure" iNPH cases, the cohort has been carefully evaluated to achieve the highest possible specificity. Unfortunately no study illustrating the diagnostic accuracy of iNPH exists. Nonetheless despite the limited nature of our patient material, this work provides insights into interesting aberrations from Aβ production typical for AD.
7 Conclusions

The occurrence of AD-like pathology in iNPH suggests that these two conditions share a similar molecular background. However, in the light of our findings, it seems that these two conditions differ with respect to events leading to the accumulation of Aβ. The expression of APP increases in iNPH, but not in LOAD, and thus it could be a more crucial factor in determining Aβ levels in iNPH as compared to LOAD. The activity of BACE1, the rate-limiting enzyme regulating the initial step in the production of Aβ, has been shown to increase in AD, whereas the activity of γ-secretase remains unaltered. Here, we showed the opposite phenomenon took place in iNPH brain samples. Especially the increase in γ-secretase activity in iNPH brain samples is interesting. Therapeutic approaches inhibiting γ-secretase have been widely explored in AD. However, these studies have shown that interfering with γ-secretase activity is problematic because γ-secretase –mediated cleavage is required for the physiological function of a large number of proteins in addition to APP and thus it may lead to adverse side-effects. Yet in iNPH, molecules restoring the normal activity of γ-secretase could be beneficial. Nevertheless, it is likely that other alterations in molecular cascades contribute to disease pathogenesis in iNPH, since the γ-secretase activity is required in both amyloidogenic and non-amyloidogenic pathways. In fact, the expression of ADAM10 was increased in iNPH, potentially translating to increased α-secretase activity. This might be a compensatory mechanism arising as a response to the increased production of APP. It is very likely that there are other yet unknown alterations taking place in iNPH pathogenesis and the accumulation of Aβ in general might be a secondary event. In both iNPH and AD, the accumulation of Aβ most likely results from an imbalance in its production and clearance. However, the emphasis in AD is in the impairment of Aβ clearance, while potentially altered production may be more crucial in iNPH.

In the present study, differences were also observed in the expression of TTR as well as in the levels of the protein in the CSF of iNPH patients. TTR is considered to be one of the main protective factors in AD and its expression as well as the actual amount of TTR protein increases in AD brain. The CSF levels of TTR correlate with AD severity. On the contrary, the expression of TTR in iNPH was less than in non-demented controls and no correlation was found with disease severity, shunt prognosis, nor brain pathology. In conclusion, this could mean a less significant role for TTR as a neuroprotective factor in iNPH. It also challenges the hypothesis that the increase of TTR seen in AD would result from mere Aβ accumulation. On the other hand, it is possible that the potential events leading to enhanced expression of TTR, such as the increased BACE1 activity in AD, is absent or even fails in iNPH, thus contributing to the accumulation of Aβ. This also reinforces the hypothesis that the underlying molecular events related to the accumulation of Aβ are different in iNPH and AD. Interestingly, APOE4 displays a positive correlation with Aβ deposition in a similar manner in iNPH and AD. On the contrary, none of the AD-associated SNPs nor the combined polygenic risk score showed a correlation with the deposition of Aβ in iNPH. The prognostic value of APOE4 is less significant in iNPH than in AD, in the latter condition it is the main risk factor in both EOAD and LOAD. In iNPH, APOE4 does not predict the condition, although it seems to contribute to the accumulation of Aβ. ApoE4 might either have additional functions in AD than in iNPH or the accumulation of Aβ caused by the presence of APOE4 is more crucial for the pathology of AD.

In summary, our results provide evidence that the Aβ deposition observed in iNPH and AD might be analogous, but the fundamental mechanisms leading to Aβ accumulation in these two disorders seem to be different. On the other hand, since these conditions
frequently co-occur, they might share some overlapping pathological steps. Nonetheless, it appears that AD and iNPH are two separate conditions and not different manifestations of same pathological events.
8 Future perspectives

The relative rarity of iNPH is at least partly the reason why it is such a poorly known condition. The fact that AD and many other neurodegenerative conditions are frequent comorbidities, also hinders the diagnostics. Nevertheless, iNPH is a condition with unique features, and more importantly, at least to some extent it is a treatable form of dementia. Although the treatment is not a final cure and only delays the disease progression, the quality of life can improve dramatically for patients with good shunt response. Thus, it would be crucial to recognise iNPH patients and this can only be achieved by improving the diagnostic accuracy.

Even though AD is a frequent comorbidity of iNPH and similar brain pathology is encountered in both conditions, our results suggest that the similarity might be only superficial or coincidental. In general, the role of Aβ in iNPH is not completely understood since not all patients with its typical clinical phenotype display Aβ pathology. Aβ might be secondary in iNPH pathology, with other yet unidentified mediators, leading to the disease phenotype. Even though Aβ pathology has been considered as an essential feature of AD already for couple of decades, it still remains obscured. The physiological role of Aβ is not known and the functions of APP have only been partially described. Additional studies into iNPH pathology might provide interesting insights into function of APP processing cascade and also lead to the discovery of diagnostic biomarkers. Due to the comorbid relationship between iNPH and AD, relevant comparative studies would be particularly valuable in understanding the pathophysiology of both conditions. In addition, it would be very interesting to clarify the role of other features important in AD, such as neuroinflammation in iNPH, since it is considered to be linked to the deposition of Aβ.

The first step to be taken in future research is the validation of these present findings. Subsequently, further studies testing our theories related to the production of Aβ in iNPH could be performed. It would be intriguing to determine whether the Aβ accumulation in iNPH truly is dependent on the surplus of APP and to elucidate the mechanisms behind the over-expression of APP. The difference in the production of TTR is also interesting and challenges the view that in AD, it is triggered solely by the accumulation of Aβ. The expression of TTR was not enhanced in conjunction with Aβ deposition in iNPH. It is probable that some yet unidentified mechanisms regulate its production in AD, but are absent or fail in iNPH. The partial differences in the role of ApoE4 are equally interesting, since a similar correlation is seen with Aβ, but not in the risk and progression of disease in general.

Finally, as already has been discussed here and in earlier studies, it is far from certain that iNPH truly is one single disease. Even though iNPH has some described diagnostic criteria, in reality, the patients are a heterogeneous group with different patterns and the severity of symptoms and various combinations of comorbidities. Perhaps in the future, iNPH can be classified with higher precision or alternatively it will become a less stringent term used to describe varying degrees of gait, cognitive and urinary symptoms. Whatever the truth, further research will be needed to find the answers.
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**TIINA LAITERÄ**

Idiopathic normal pressure hydrocephalus (iNPH) is a rare condition with typical symptoms. The accumulation of β-amyloid (Aβ) plaques typical for Alzheimer’s disease (AD) is seen in iNPH as well, yet the underlying mechanisms are unknown. This thesis deciphers the accumulation of Aβ in iNPH on the level of protein processing as well as by investigating underlying gene expression. The findings presented in this thesis provide new information considering the formation of Aβ plaques in iNPH and reveal several interesting differences in molecular pathology between iNPH and AD.