Islet amyloid polypeptide indeed expressed in the human brain?

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Abstract

Aims: This study aims to study the association between pancreatic islet amyloid polypeptide (IAPP) and Alzheimer’s disease neuropathological change (ADNC) in brain biopsies obtained from subjects with idiopathic normal pressure hydrocephalus (iNPH) and in post-mortem (PM) brain samples obtained from aged individuals.

Methods: For the immunohistochemical (IHC) analyses, two IAPP antibodies (Abs), monoclonal and polyclonal, and Abs directed towards ADNC were applied.

Results: The iNPH cohort included 113 subjects. Amyloid-β (Aβ) was detected in 50% and hyperphosphorylated τ (HPτ) in 47% of the cases. Concomitant pathology was seen in 32%. The PM cohort included 77 subjects. Aβ was detected in 69% and HPτ in 91% of the cases. Combined Aβ/HPτ pathology was seen in 62%. Reactivity for the monoclonal IAPP was not detected in the brain tissue in either of the cohorts. Reactivity for the polyclonal IAPP was observed in all 77 PM brain samples.

Conclusions: There was no specific expression of IAPP in human brain tissue; hence, an association between IAPP and ADNC is not assessable. Of note, the observed reactivity of the polyclonal IAPP Ab was not reproduced with a specific monoclonal Ab; thus, we considered the observed staining with the polyclonal Ab to be unreliable. When using IHC, several pitfalls, especially the choice of an Ab, always need to be considered. Polyclonal Abs cross-react with other epitopes and proteins, thus leading to false-positive results. This seems to be the case for the polyclonal IAPP Abs in the human brain.

KEYWORDS
Alzheimer’s disease, Alzheimer’s disease neuropathological change, amylin, beta-amylloid, idiopathic normal pressure hydrocephalus, islet amyloid polypeptide, type 2 diabetes mellitus

INTRODUCTION

Alzheimer’s disease (AD) is the most common clinical diagnosis of dementia. Age, sex, educational level, apolipoprotein ε4 status, and cardiovascular disease are some risk factors associated with AD [1]. Several epidemiologic, pathophysiologic, and molecular studies also associate type 2 diabetes mellitus (T2DM) with AD [2–4]. This association has not been confirmed when assessing neuropathology in large cohorts of aged individuals [4–7].

Islet amyloid polypeptide (IAPP), also called amylin, is a hormone synthesised and secreted by pancreatic β-cells [8, 9]. IAPP is involved in insulin and glucagon homeostasis, regulation of bone mass, and blood pressure as well as satiety and gastric emptying. In pathological conditions, IAPP is the main component of islet amyloid, contributing to β-cell loss and finally T2DM. This is due to the molecular structure of IAPP and its ability to form β-sheets that aggregate into amyloid fibrils [8–10]. These features are also common to other amyloid-forming proteins in humans [10].

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Amyloid β (Aβ), one of the elements of AD neuropathological change (ADNC), is prone to aggregate within the grey matter of the brain as well as in the meningeal and cortical blood vessels [11]. The level of Aβ pathology is not associated with cognitive impairment, as it can be detected in the brains of young subjects as well as in aged individuals that do not experience cognitive decline and dementia [6, 12, 13].

During the last decade, several groups have studied the association between Aβ and IAPP, as both proteins show amyloidogenic properties and are primarily detected in the aged [2, 14]. Molecular interaction and the cross-seeding mechanism between Aβ and IAPP have been described in in vitro studies and animal models [15–17]. Proximity ligation assays and immunohistochemical (IHC) studies have co-localised the Aβ and IAPP in post-mortem (PM) human brains [15, 18, 19].

Surprisingly, some studies describe associations between IAPP and hyperphosphorylated τ (HPτ), the other hallmark lesion of ADNC [19–21]. The presence of Aβ and HPτ deposits in pancreatic β-cells has also been described in a few studies but has not been reproduced by others [15, 19, 22, 23].

Idiopathic normal pressure hydrocephalus (iNPH) is a neurological condition, affecting older individuals, caused by altered cerebrospinal fluid (CSF) circulation, presenting with cognitive impairment, gait disturbances, and urinary frequency and incontinence. The only treatment strategy available is a ventriculoperitoneal shunt (VPS) insertion that normalises the CSF flow and reverses the symptoms [24]. During such curative surgery, a brain biopsy can be obtained from the area of the shunt channel. Numerous studies describe ADNC in biopsies from iNPH patients; moreover, when present, it is associated with a worse shunt response and progression to AD [25–30].

The aim of this study was to assess the presence of IAPP in brain tissue obtained during VPS insertion from iNPH subjects with and without ADNC. This allowed us to assess the protein content in the human brain tissue without changes caused by the agonal state, PM delay (PMD), or a long fixation time. These results were compared with those observed while assessing the pathology in PM brain tissue from aged subjects with or without ADNC.

MATERIAL AND METHODS

Study subjects

iNPH subjects

The brain biopsies were obtained from the right frontal lobe during curative VPS insertion as previously described [24, 28, 30]. The specimens were fixed in 10% buffered formalin (4% formaldehyde) at room temperature for 24–72 h. Thereafter, the samples were processed into paraffin blocks and sectioned into 4 μm thick sections for haematoxylin–eosin (HE) staining and H&C stains.

In total, 120 subjects, biopsied at Uppsala University Hospital (UUH) from 2019 to 2021, were identified in the diagnostic database of the Surgical Pathology Department of the UUH. Seven cases lacked grey matter in their biopsy; thus, there were 113 cases for analysis.

Post mortem samples

Four tissue microarray (TMA) blocks were constructed from 77 PM brains that had undergone standardised neuropathological examination at UUH during 2009–2016 [31]. The subjects included those displaying none or various levels of Aβ and/or HPτ in their brains. Two samples, measuring 2 mm in diameter, were obtained from the amygdala of each brain following a previously described method [32]. The TMA blocks were cut into 4 μm thick sections for further analysis.

To validate the protocols for the IHC stains while using IAPP antibodies (Abs), a tissue sample was obtained during the autopsy from the pancreas of an 85-year-old male.

Immunohistochemistry

The IHC stains were performed using the automatic platform, Dako Autostainer Plus (Agilent Technologies, Santa Clara, CA, USA) with the Dako EnVision Flex detection system according to the manufacturer’s instructions. The Abs and the pretreatment strategies used are summarised in Table 1.
Assessment of the samples

All samples were assessed using light microscopy at ×20 to ×400 magnification. The pathology within a sample was assessed and dichotomised as present or not, except for the polyclonal IAPP Ab, where the compartmentalisation of the staining was also noted.

Statistical analysis

The statistical analyses were performed using IBM SPSS statistic software, version 28 (IBM Corp., NY, USA). To describe the cohort, means and standard error of means (m ± SE) were used. Non-parametric test, Mann–Whitney U test (MWU), was applied to study the differences between the groups. The correlations between the different variables were defined using a non-parametric Spearman’s rho two-tail test.

RESULTS

The IAPP Abs were tested on PM pancreatic tissue from an 85-year-old male with cardiovascular disease, cerebrovascular disease, renal failure, and a clinical diagnosis of dementia but no known T2DM. The cause of death was cardiac arrest. The PMD was 24 h. The pancreas displayed extensive autolytic changes, with only a few viable β-cell islets. Immunoreactivity for both monoclonal and polyclonal IAPP Abs was seen in the pancreatic β-cells; however, the polyclonal Ab also displayed background staining, as illustrated in Figure 1.

Post-mortem samples

There were four TMAs, which included core samples (CS) from the amygdala of 77 subjects, 32 females and 45 males, with an age range at death of 50 to 100 years, m ± SE 75.79 ± 10.02. The PMD varied from 24 to 144 h, and the fixation time was less than 14 days. In total, 53 cases displayed various levels of ADNC, whereas 18 displayed HPτ only, corresponding with primary age-related tauopathy. Cerebral amyloid angiopathy (CAA) was seen in 27 cases.

Within the samples from the amygdala, Aβ pathology was seen in the CS from 53 subjects (68.8%), of which 71.9% were females and 66.7% were males. No significant difference was seen in the Aβ expression between the sexes (MWU, p = 0.300). The extent of the pathology varied within the CS, from a single aggregate to multiple aggregates.

CAA was present in nine subjects (11.7%). HPτ pathology was observed in 70 subjects (91.0%), including all females and 84.4% of the men. There was a significant difference in the expression of HPτ between the sexes (MWU, p = 0.020). The extent of the pathology varied within the CS, from a few neurites to full-blown pathology, with numerous tangles and neuropil filled with HPτ reactive threads.

Concomitant Aβ and HPτ pathology was seen in 48 cases (62.3%), of which 71.9% were females and 55.6% were males.

When applying the polyclonal Ab, IAPP reactivity was seen in all CS. Intracellular IAPP labelling of granular structures was seen in all samples, whereas extracellular, ‘plaque-like’, aggregates were seen in 22 subjects. Furthermore, immunoreactivity in the vascular

### Table 1: Immunohistochemical stains.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Company/code</th>
<th>Dilution</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloid β</td>
<td>4G8</td>
<td>Biolegend/800703</td>
<td>1:1000</td>
<td>98%–100% FA−2 min</td>
</tr>
<tr>
<td>Islet amyloid polypeptide (IAPP)</td>
<td>R10/99</td>
<td>ThermoScientific/MA134685</td>
<td>1:200</td>
<td>CB and 80% FA−5 min in ac</td>
</tr>
<tr>
<td>IAPP</td>
<td>Polyclonal</td>
<td>Biozol/T4149</td>
<td>1:700</td>
<td>Target Retrieval solution high pH, 80% FA−5 min</td>
</tr>
<tr>
<td>Hyperphosphorylated (Ser202/Thr205) (TAU8)</td>
<td>PHF-TAU-AT8</td>
<td>Fisher Scientific-Invitrogen/MN1020</td>
<td>1:1000</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ac, autoclave; CB, citrate buffer pH 6.0; FA, formic acid.
compartment was seen in 38 samples. Examples of the staining pat-

tterns are shown in Figure 2A–C. The plaque-like IAPP labelling was

seen, independent of the presence of A\(\beta\) or HP\(\tau\) in the CS, that is, no

significant correlation for A\(\beta\) (\(p = 0.318\), Spearman’s rho = 0.1) or

HP\(\tau\) (\(p = 0.387\), Spearman’s rho = −0.1). IAPP immunoreactivity in

the vessel walls was seen, independent of the presence of CAA, that

is, no significant correlation (\(p = 0.071\), Spearman’s rho = 0.2). Con-

trary to the above, no IAPP reactivity was seen in any of the samples

when using the monoclonal IAPP Ab, as shown in Figure 2D.

iNPH subjects

In total, 113 samples from the iNPH subjects were included in

this cohort, including 44 females and 69 males. At the time of the

biopsy, the age range was 54 to 86 years with the \(m \pm SE\) 73.93

\(\pm 6.18\) years in total, specifically 74.86 \(\pm 4.51\) years in females and

73.33 \(\pm 6.90\) years for males. No significant age difference was seen

between the sexes (MWU, \(p = 0.354\)).

Light microscopic assessment of the samples verified the pres-

ence of grey matter in the biopsies and the compartmentalisation of

the IHC stains. A\(\beta\) was detected in 56 subjects, representing 49.6% of

the cohort, 61.4% of the females and 42.0% of the males, with a sig-

nificant difference (MWU, \(p = 0.046\)). The pathology varied from a

single aggregate to multiple aggregates. CAA was seen in six biopsies

(5.3%). HP\(\tau\) was seen in 53 subjects, 46.9% of all subjects, 40.9% of

the females and 50.7% of the males, with no significant difference

(MWU, \(p = 0.310\)). The majority of the HP\(\tau\) reactive cases displayed

sparse pathology with a few grains and neurites; however, in 11 cases

(20.7%), more extensive tangle and/or neuritic pathology was seen.

Concomitant A\(\beta\) and HP\(\tau\) pathology was seen in 36 cases (31.9%),

16 females and 20 males. No IAPP reactivity was seen in the biopsies

using the monoclonal Ab, as shown in Figure 2E.

DISCUSSION

Several studies have suggested an association between T2DM and

AD in vitro, in animal models and in PM human tissue [15–19]. Here,

for the first time, we assessed the expression of the pancreatic protein

IAPP in surgical brain biopsies obtained during a curative shunt

insertion procedure from iNPH subjects with or without ADNC. Ad-

ditionally, we evaluated the IAPP expression in PM brain samples

from the amygdala region, from 77 subjects with or without ADNC.

In our iNPH cohort, including subjects between 54 and 86 years

old, A\(\beta\) was present in 50% and HP\(\tau\) in 47% of the cases, and com-

bined A\(\beta\)/HP\(\tau\) pathology was seen in 32% of the cases. The frequency

of the pathology is congruent with what has previously been

described in the majority of iNPH cohorts [28–30, 33]. These results,

however, are lower compared to our previous studies due to the dif-

terent assessment strategies and the selection criteria of the cohort

[27, 34].

In the TMA, including 77 CS from the amygdala, A\(\beta\) pathology

was detected in 69% of the cores and HP\(\tau\) in 91%. This is in line with
the stepwise progression of the pathologies, as the Aβ primarily affects the cortical regions of the brain; when seen in the amygdala, the pathology has already reached Thal phase 2 [11]. In contrast, the HPr can be seen in the amygdala early on in the disease process and can be detected in younger individuals [13, 35].

We assessed the immunoreactivity of two different IAPP Abs, one monoclonal (clone: R10/99) and one polyclonal (T4149), with the latter being used in previous studies assessing PM brain [18, 19]. The performance of these Abs was tested on pancreatic autopsy tissue. Despite a defined agonal state due to cardiac arrest and a PMD of 24 h, the pancreas displayed extensive autolysis, with only a few remaining β-cell islands. Both Abs labelled pancreatic islands. The polyclonal Ab also displayed background staining. The agonal state is a factor to consider when assessing PM tissue, as prolonged death is associated with a lower pH, hence increased autolysis [36]. Post-mortem, the body’s organs undergo autolysis caused by the deposition of proteolytic enzymes. The pancreas is recognised as undergoing autolysis soon after death, as it is rich in digestive enzymes, resulting in morphological changes and altered protein expression as judged by IHC [37, 38]. In line with the literature, our case displayed marked autolysis within the pancreatic islet 24 h after death [37]. In Sweden, an autopsy cannot be performed without consent from a close relative; thus, the PMD is often 1 day or more. The information regarding the agonal state has not been provided in previous publications, and PMD is only given in one study assessing the IAPP pathology in the pancreas and brain [18, 19].

No IAPP pathology was seen within the brain tissue in either the iNPH or the PM cohorts when using the monoclonal IAPP Ab. Of note, when applying the polyclonal IAPP Ab, different expression was seen in various cellular compartments and structures within the PM brain tissue. In line with what has been reported previously, the IAPP labelling was seen as granular structures in the neuronal soma, extracellular plaque-like aggregates as well as inclusions in the vessel walls [15, 18, 19]. Since we could not detect any specific IAPP expression with the monoclonal Ab, the positive structures visualised by the polyclonal Ab have to be interpreted as non-specific.

IHC is used daily in research and clinical diagnostics [39, 40]. There are numerous pitfalls to consider when using IHC. Factors such as PMD/cold ischaemia time, type of fixation, fixation time, type of paraffin, storage time, section thickness, antigen retrieval technique, mode of staining (manual or automatic), and detection system should be considered when performing and assessing the IHC staining outcome [41, 42]. Additionally, the choice of an antibody is crucial. Monoclonal Abs are highly specific, as they recognise only one epitope on an antigen, thus reducing the risk of non-specific binding and hence immunoreactivity. In contrast, the polyclonal Abs recognise several epitopes, resulting in a higher affinity to an antigen but also non-specific staining caused by binding to similar epitopes leading to what is in general known as cross-reactivity [41].

In a study from 2021, Rees and colleagues revealed strong cross-reactivity of several polyclonal IAPP Abs with a neuropeptide, calcitonin gene-related peptide (CGRP), which is widely expressed in the brain and shares 40%–50% of the amino acid sequence with IAPP [43]. Previous studies assessing the IAPP expression in PM brain have used polyclonal IAPP Abs [15, 18, 19]. The polyclonal IAPP Ab used by us and in the previous studies on this topic (T4149, PeninsulaLaboratories) recognises the full length of the IAPP protein, thus including the sequence recognised in CGRP leading to labelling that is interpreted, by us, as non-specific [43]. Of note, the monoclonal IAPP Ab used by us (clone R10/99) recognises an 11-amino acid sequence (7–18) of the IAPP protein.

We assessed the performance of a monoclonal Ab, which provides specific labelling of IAPP. IAPP monoclonal Ab staining was observed in the pancreas as expected. However, there was no staining at all in the surgical brain biopsies, which lacked the pre-analytical alterations caused by PMD and long fixation time. IAPP labelling with the monoclonal was also absent from the PM brain samples, altered by various pre-analytical factors. The ADNC was detected in both our cohorts and, according to our results, did not influence the IAPP immunoreactivity of either of the Abs used. As polyclonal Abs are more unreliable, the expression seen in our PM cohort was interpreted as non-specific. Thus, based on our results, we cannot confirm that specific IAPP expression is observed in brain tissue or vascular structures, as previously reported.

IAPP, like insulin, can cross the blood/brain barrier, but a seeding mechanism of pathologic IAPP protein from the blood/plasma to the brain was not confirmed in an animal model, further questioning the reported role of IAPP pathology within the brain [44, 45].

Several studies performed in vitro or in animal models have suggested a connection between AD and T2DM and have reported an association between IAPP and Aβ or even in a single case with HPr [2, 16, 17, 21, 46]. These studies are interesting on a molecular level, as interactions between the different altered proteins promoting the pathological processes are increasingly recognised in neurodegenerative diseases [47]. IAPP is an amyloidogenic protein, prone to aggregate and with a similar structure to other amyloids. This makes it an interesting protein to study with regard to protein alteration and seeding [10]. The connection between IAPP and ADNC has been seen in vivo studies as well as in animal models and the PM human brain, albeit using the polyclonal IAPP Abs; thus, the results are not reliable [15, 16, 18, 19, 46].

The association of T2DM and a clinical diagnosis of dementia of AD type has also been suggested in several epidemiological studies of aged individuals. However, this association has not been confirmed in large neuropathological cohorts when a definite diagnosis of the pathological type of dementia was available [4–7, 14, 48]. One explanation for the outcome might be the cognitive decline caused by mixed pathologies that increases with age and is not acknowledged until the final PM examination of the brain is carried out [6, 47, 49]. T2DM is associated with vascular disease and vascular changes within the brain, but it has not been associated with ADNC in numerous PM studies [4–7, 14]. One study revealed that the only brain proteinopathy associated with pancreatic IAPP in T2DM subjects was transactive DNA binding protein 43, the cause of limbic-predominant age-related TDP encephalopathy [23, 50]. This is certainly intriguing as it connects T2DM with cognitive impairment and brain pathology.
In summary, we had the opportunity to assay the expression of IAPP in surgical brain biopsies of 113 iNPH subjects. This is unique, as most studies regarding IAPP in the brain are performed on PM tissue, animal models or in vitro. Here, we could assess protein expression in the human brain without pre-analytical confounding factors affecting the epitopes to be labelled. We also assessed the IAPP in a cohort including 77 PM samples from the amygdala region, as previous studies have been performed on PM brains. Both cohorts included samples with and without ADNC. There was no immunoreactivity for IAPP in either of the cohorts when using the monoclonal IAPP Ab. In contrast, the IAPP protein reactivity was visualised in different structures within the brain samples when using the polyclonal Ab, in line with what has been reported in previous publications [15, 18, 19]. We interpreted the reactivity of the polyclonal IAPP Ab as non-specific due to the total lack of IAPP reactivity while using the specific monoclonal Ab. Of note, polyclonal IAPP Abs have been reported to cross-react with a neuronal protein, leading to false-positive outcomes [43].

In conclusion, we could not verify any association between ADNC and IAPP in brain tissue in either of our cohorts; hence, there seems to be no support for a causative association between ADNC and T2DM, in line with previous neuropathological studies [4–7]. An association has been reported based on molecular studies and in animal models, but not when studying human brain tissue. A significant factor leading to a false-positive outcome may be the choice of the Ab. A reliable, specific Ab is crucial when assessing a surgical specimen as the interpretation of the outcome can affect the final diagnosis and thereby the choice of treatment. To produce reliable results, pre-analytical and analytical variables have to be taken into consideration [41].

AUTHOR CONTRIBUTIONS

Conceptualisation and methodology; Sylwia Libard and Irina Alafuzoff. Investigation: Sylwia Libard. Writing: Sylwia Libard and Irina Alafuzoff. Both authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to report.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/nan.12917.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The study has been approved by the regional Ethical Committee of Uppsala, Sweden: for the post-mortem tissue, approval number, #2011/286, updated 2015, and for the biopsies, #2013/176, updated 2016. All iNPH subjects included have given their informed consent for the use of diagnostic tissue for scientific purposes.

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