Review report on the PhD thesis submitted to the Medical University of Bialystok

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Title: The assessment of selected proteins related to synaptic plasticity in Alzheimer's disease

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The project background

Alzheimer's disease (AD) is a growing concern globally, affecting over 55 million people directly, their families and the community at large. AD accounts for over 60% of all dementia cases worldwide, being the most common form of dementia. Alzheimer's disease (AD) continues to present urgent strains on clinical care, public health efforts and palliative care. Although the ultimate confirmation of AD pathology is by autopsy examination of brain tissue for extracellular amyloid plaques made of amyloid-beta (Aβ) peptides and intraneuronal neurofibrillary tangles (NFTs) containing phosphorylated tau (p-tau) forms, *in vivo* diagnosis is presently achieved by using either cerebrospinal fluid (CSF) or neuroimaging biomarkers. Neuroimaging biomarkers that can jointly identify biological evidence of AD include Aβ positron emission tomography (PET) for brain amyloidosis, tau-PET for NFT pathology, structural magnetic resonance imaging (MRI) for hippocampal atrophy, and fluorodeoxyglucose (FDG) PET for brain metabolic changes. For CSF, three markers (referred to as the core AD biomarkers) can jointly detect "a positive AD profile". These are: Aβ42 (or Aβ42/Aβ40 ratio), which reflects Aβ plaque pathophysiology; phosphorylated-tau (p-tau), an indicator of tau phosphorylation; and total-tau (t-tau), a neuronal injury or neurodegeneration marker. The concentrations of these biomarkers change in individuals with biological evidence

of AD compared with normal controls. Aβ42 is decreased and Aβ40 is unchanged. However, the Aβ42/Aβ40 ratio adjusts for inter-individual differences in the concentrations of the aggregation-prone Aβ42 peptide, making the ratio a more reliable indicator of Aβ plaque pathology compared with Aβ42 alone. P-tau and t-tau levels are both increased in AD versus unaffected controls, with the biomarker concentrations increasing according to disease severity. CSF t-tau is excellent for differentiating AD from healthy controls. CSF neurofilament light (NfL) is another strong indicator of neurodegeneration that can in principle substitute for t-tau in AD, however, unlike t-tau, CSF NfL is already increased in other neurodegenerative diseases.

The introduction

The thesis set out with an introductory description of the aforementioned basic concepts of AD. The author went on to explain the importance of synaptic pathology, the pathophysiological mechanisms involved, the role of Aβ and tau, and novel candidate synaptic integrity markers in CSF. These included neurogranin, neuronal pentraxin receptor (NPTXR), and fatty acid binding protein 3 (FABP3). For each of these markers, the author provided Sankey plots and gene ontology terms describing the biological processes that the analyte is involved in. The main hypothesis was that synaptic degeneration is a key and early signal of AD and the indicated markers (neurogranin, NPTXR and FABP3) may serve as clinically useful markers for their detection and quantification.

The introduction starts from page 7 to page 25. The provided information is carefully referenced with relevant and recent literature, mostly published the past decade in highly valued peer-reviewed journals.

Aims

There were five main aims:

1. To evaluate the concentrations of neurogranin, NPTXR and FABP3 in CSF of AD, MCI and non-AD patients;
2. To compare the levels of the synaptic protein markers between diagnostic groups;
3. To correlate the markers between themselves and with the classical/core AD markers in CSF, as well as clinical assessments of cognition;
4. To evaluate the diagnostic potential of the synaptic markers; and
5. To assess bioinformatic relationship of the biological processes involved in synaptic pathology in AD.

The aims are concisely formulated and presented. The importance of the aims in the research field cannot be overstated. AD scientists have come to appreciate that synaptic degeneration is a key part of the disease process seen in AD, and can explain many of the pathophysiological changes observed in the disease.

Materials and methods

CSF samples were collected from patients attending the neurology clinical at the Jagiellonian University Medical College. The participants included 70 individuals; 34 AD, 18 MCI and 19 non-demented controls. The CSF collection procedure is described, so is the diagnosis and classification of patients using MRI and CT scans as well as CSF core biomarkers.

Next, details of the immunoassay platforms, methods and the reagent batches used to measure the core biomarkers and the synaptic markers of interest in CSF are presented.

The statistical analysis used to evaluate the data obtained are also described.

Ethical clearance certificate is provided at the end of the thesis.

Results and Discussion

It is impressive that the study results have been published in four peer-reviewed articles: two each in the International Journal of Molecular Sciences and the Journal of Clinical Medicine.

Publication P.1 is a systematic review of the value of neurogranin and VILIP-1 (the latter marker was not a focus of the PhD thesis) as indicators of neurodegeneration in AD.

Publication P.3 is an original article that demonstrates that FABP3 and *APOE* e4 are lipid metabolism markers in AD.

Publication P.4 is also an original article that shows that neurogranin and NPTXR are synaptic dysfunction markers in AD.

Publication P.5 combined CSF biomarker and bioinformatic analyses to evaluate markers of synaptic and axonal dysfunction in AD.

The author went on to describe the key results from each of the publications, and also provided summary discussion of the results and their implications.

Printouts of the publications were also included in the thesis.

The % contribution of the author to each of the articles is also noted at the end of the thesis.

In each article, the results of the study are meticulously presented – in the form of interactive, and colour-guided figures and tables, and discussed.

A final abstract for the thesis is provided in both Polish and English.

Specific comments

It is important to note that the research thesis has no significant flaws. Nonetheless, there are a few points that should be addressed:

1. In Publication P.5., the authors used the classifications AD, MCI and control. It is important to differentiate between MCI and AD here – if AD is used to mean “AD dementia” and if MCI refers to MCI altogether or “MCI due to AD”
2. It will be useful to add a final concluding statement for the whole thesis at the end, just before the paper out prints.

Final remarks

The thesis is of world-class quality. The conclusions are supported by the results, and the discussions are clearly suited to the results. Furthermore, all of the study aims set out at the start of the research have been answered. The language is clear, coherent and comprehensive. I fully believe that this dissertation complies with Polish and international standards and requirements for doctoral education. It is demonstrable that Maciej has contributed original solutions to a known scientific problem. He was shown enviable theoretical and practical knowledge in the areas of biomarkers and bioinformatics. In conclusion, the author has the capacity to independently formulate research questions and conduct hypothesis-driven research to address them.

I therefore strongly recommend that the Senate of the Medical University of Bialystok moves this thesis further in the process towards awarding a doctoral degree, which may include a public defense. This is a distinguished dissertation by all standard.

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