PERSPECTIVE

Cerebrospinal fluid neurogranin as a new player in prion disease diagnosis and prognosis

Neurogranin (Ng) and its role as Alzheimer's disease (AD) biomarker: Ng is a calmodulin-binding protein mainly expressed in cerebral structures such as the cortex, hippocampus and striatum. It is mainly located in the dendritic processes, particularly in post-synaptic compartments, but also in the cytosolic compartment, being likely involved in the regulation of the intracellular calcium-calmodulin signaling pathway (Represa et al., 1990). In the last decade, a plethora of studies have demonstrated that cerebrospinal fluid (CSF) Ng is increased in AD patients and in individuals with an AD-like CSF profile (Kester et al., 2015a). This increase seems to be disease-specific because other neurodegenerative conditions including frontotemporal dementia, Lewy body dementia, Parkinson's disease, progressive supranuclear palsy, multiple system atrophy or Huntington's disease, present CSF Ng concentrations similar to controls (Wellington et al., 2016). Ng levels in CSF appear to be elevated in mild cognitive impairment (MCI)-affected individuals who progress to AD and are highly related to memory and cognitive function (Kester et al., 2015a; Tarawneh et al., 2016), which indicates that this protein may serve as an early AD biomarker with diagnostic utility in pre-dementia disease stages, and with prognostic utility to predict cognitive decline and MCI-to-AD conversion.

These findings, together with the prominent presence of Ng in post-synaptic locations, suggest that high CSF Ng concentration reflects synaptic degeneration even at pre-symptomatic stages. Indeed, the fact that Ng is reduced in the AD brain (Kvartsberg et al., 2019), probably due to loss of synaptic density, indicating that the high levels of this protein in the CSF could be originated from leaking amounts filtered to CSF upon brain synapse disintegration. In addition, considering that synaptic dysfunction is common in many neurodegenerative disorders, AD-specificity of high CSF Ng levels puts forward the hypothesis that there is an underlying mechanism particularly associated with the AD pathology. In this sense, the clear correlation between CSF Ng and other AD biomarkers, especially total-tau and phosphorylated-tau (Kester et al., 2015b; Portelius et al., 2015; Tarawneh et al., 2016; Wellington et al., 2016), indicates that Ng secretion from neurons can be related to the AD tauopathy process occurring in the brain.

We recently studied the presence of Ng in the CSF and brain tissue of Creutzfeldt-Jakob disease (CJD) and AD patients. CJD is the most prevalent form of prion disease in humans, which manifests itself as a rapidly progressive dementia with very short survival time. Despite the partial clinical overlap with AD, particularly at early disease stages, the etiology of CJD is found in the abnormal conversion of the prion protein into pathogenic forms, which induces massive neuronal destruction leading to spongiosis. In contrast, AD pathology is related to processes known as amyloidosis and tauopathy that invade and damage neuronal structures, but it is impossible to identify what exactly the primary cause of the disease is.

In our study, we obtained surprising findings that may change some of the established concepts regarding CSF Ng: i) the highest levels are found in CJD (over those found in AD); ii) this biomarker offers a promising role in CJD prognosis; and iii) it cannot be considered as a specific marker of synaptic degeneration, but rather a marker of neuronal damage (Blennow et al., 2019).

Ng in CJD: We recently investigated the role of Ng as a biomarker for CJD in comparison to AD (Blennow et al., 2019). In agreement with the literature, we found increased CSF Ng concentration in AD compared to controls. However, higher levels of CSF Ng were observed in CJD (2.5 fold change with respect to AD). We also reported significantly low Ng levels in the cortex and hippocampus of CJD compared to AD and control (**Figure 1**). Collectively, these results indicate that CSF Ng is not a specific AD marker, and do not support the theory that Ng is transferred to the CSF due to any AD-specific mechanisms.

We studied the potential to use CSF Ng as a diagnostic biomarker for prion disease. We found an area under the curve derived from receiver operating characteristic curves (AUC) of 0.96 for CJD *vs.* neurological controls and of 0.85 for CJD *vs.* AD (Blennow et al., 2019). These values are far better than the AUC we found for AD *vs.* controls (0.73), which is in good agreement with previous publications (Tarawneh et al., 2016). Despite the good accuracy of CSF Ng in the differentiation of CJD *vs.* controls, this was slightly lower than the accuracy reported for tau and 14-3-3, two gold standard CSF biomarkers for CJD; but superior to the accuracy detected for CSF neurofilament light (NFL), a recent prion disease biomarker.

Interestingly, CSF Ng displayed significantly positive correlation with tau, but not with NFL. This is explained by the fact that NFL is mainly expressed in the axons of the white mater region, where Ng staining was undetectable in our cases. In our study, we demonstrated that Ng is not only expressed in post-synaptic compartments, but also in the neuronal body, showing positive correlation with tau, synaptophysin and PSD-95 protein levels in CJD brain tissue. These results challenge the current view of Ng as a sole marker of synaptic damage and suggest a general role as a marker of neuronal damage, which can explain the strong correlation between CSF Ng and tau observed by us and many others. Indeed, positive correlation between CSF Ng and tau has been observed in many conditions, including controls, tauopathies and synucleinopathies, among other diagnostic groups (Portelius et al., 2018).

Considering the fact that CJD-affected brains present synaptic and axonal damage to a major extent than AD-affected brains, we can conclude that CSF Ng mirrors massive destruction of neuronal structures in the brain, which occurs at early CJD stages likewise in AD. Similarly to what has been described in AD, we found an inverse correlation between CSF Ng and brain Ng at disease-specific level (controls, AD and CJD) and at CJD subtype-specific level. Regarding the latter, we observed higher CSF Ng concentration in CJD MM1 cases than in VV2 cases, together with a stronger decrease of brain Ng levels in MM1 compared to VV2. Thus, the inclusion of prion disease cases, which present an extreme degeneration of brain molecular and cellular structures, is essential when studying surrogate disease biomarkers in order to understand the pathophysiological underpinnings of the regulated molecules in biological fluids. This can also be applied to inflammatory biomarkers, due to the massive neuroinflammatory profile observed in CJD brain tissue. For instance, YKL-40 was initially regarded as an AD-specific biomarker; a notion that was later on refuted by works demonstrating high CSF YKL-40 in prion diseases.

Regarding biomarker confounders, CSF Ng is not affected by age or sex (Kvartsberg et al., 2015b; Blennow et al., 2019) but it displays dependency on disease subtype. In typical AD, CSF Ng levels are higher compared to atypical AD (Wellington et al., 2018). In CJD, we found that CSF Ng is increased in MM1/MV1 molecular subtypes compared to VV2 subtype, whose cortical pathological affectation is less exacerbated than that in MM1/MV1 cases (Blennow et al., 2019). This points once more towards the mirroring effect that CSF Ng entails regarding brain neuronal degeneration. Albeit we could not observe a significant increase of CSF Ng concentration along CJD progression, we found an association between CSF Ng and survival time. Compared to tau and NFL (both previously proposed as CJD prognostic biomarkers), Ng was able to explain more of the variability in CJD duration, unveiling a potential role in disease prognosis (Blennow et al., 2019). CSF Ng prognostic ability has also been demonstrated in cogni-

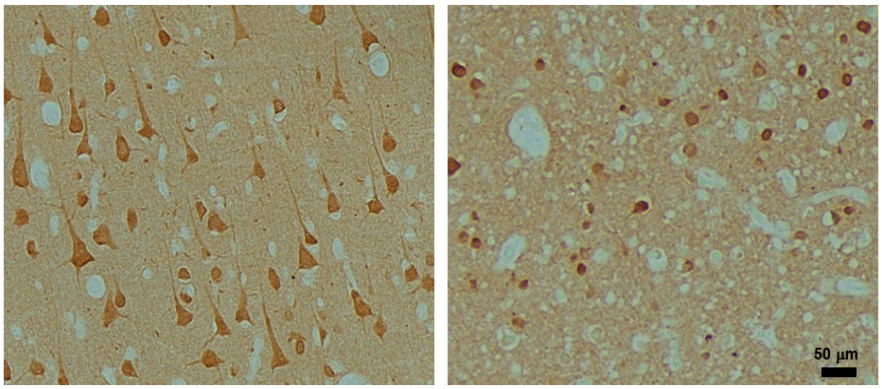

Figure 1 Immunohistochemical analysis of Ng expression in the cerebral cortex of a control (left) and a CJD (right) case.
CJD cases were characterized by a significant reduction of neural Ng immunostaining and presence of characteristic spongiform degeneration. CJD: Creutzfeldt-Jakob disease; Ng: neurogranin.

tively normal individuals in whom it can predict future cognitive impairment, and in AD patients in whom it can predict cognitive deterioration (Portelius et al., 2015; Tarawneh et al., 2016). In addition, a weak correlation was observed between CSF Ng and disease duration in AD (Wellington et al., 2016). Altogether, due to the strong correlation between neuronal integrity and cognitive function, CSF Ng can be regarded as an early marker of disease, reporting one of the initial main pathological events and predicting major disease phenotypic hallmarks (cognitive decline and dementia).

Conclusions and future perspectives: Although it is indubitable that CSF Ng distinguishes AD from controls, high levels found in CJD challenge the role of Ng as diagnostic marker when put in the differential diagnostic context. Indeed, the clinical overlap between CJD and AD, particularly in rapidly progressing forms of AD, hinders a reliable use of CSF Ng as an AD diagnostic biomarker. Up to date, rapid cognitive decline has been associated with high Ng levels, but only in MCI individuals (Kvartsberg et al., 2015a; Portelius et al., 2015). Our pilot analysis indicated that CSF Ng levels in rapidly progressing forms of AD are similar to the levels found in AD (Blennow et al., 2019). Although this result suggests that CSF Ng preserves the capacity to discriminate between CJD and AD in cases of rapidly progressive dementia, further investigation in larger cohorts is necessary to validate this finding.

Our data in CJD and previous literature in AD, allow to envisage a probable more interesting performance of CSF Ng in disease prognosis than diagnosis. This conclusion highlights the necessity to take into account different contexts of use when studying biomarkers, as they can be qualified for specific purposes depending on their performance. Considering the prognostic value of CSF Ng, it seems promising to conduct future studies on the capacity of CSF Ng to monitor disease progression and drug response in potential therapeutic interventions. For this purpose, a further characterization of Ng as prognostic marker of CJD is indispensable, which needs to include confounders such as age, sex and genetic background (in CJD, codon 129 of prion protein gene is particularly relevant) and correlations with distinct disease outcome measures.

Our study also underlines the importance of conducting parallel neurological and neuropathological studies in the field of disease biomarkers. On the one hand, the analysis of biomarkers in body fluids enables to characterize their potential use as a disease diagnosis or prognosis marker. On the other hand, their characterization in the brain tissue is indispensable to understand how biomarkers are regulated in the fluids and along the patho-physiology of the disease. To obtain the complete picture, it is equally important to conduct the analyses in a regional- and temporal- manner and in different disease subtypes. In the case of Ng, our simultaneous investigation unravelled disease-specific (AD *vs.* CJD) and disease subtype-specific (CJD MM1/MV1 *vs.* CJD VV2) correlations between Ng levels in the brain and in the CSF, as well as correlations between Ng and other neuronal proteins in the CJD brain tissue (Blennow et al., 2019).

Additionally, Ng has been reported to be mainly present in the CSF as C-terminal fragments, instead of the full length polypeptide (Kvartsberg et al., 2015a). The proteolytic cleavage of Ng is done, at least partially, by calpain-1 and prolyl endopeptidase (Becker et al., 2018), which suggests that increased levels of Ng in the CSF serve not only as a surrogate marker of neuronal damage, but can also report on the activity of proteolytic enzymes that are relevant in neurodegenerative conditions. In this regard, calpain-1 over-activation has been reported both in AD and in CJD. Thus, further investigation of the specific Ng truncated forms that populate the CSF and the brain tissue in neurodegenerative disease, will likely contribute to the understanding of the pathological mechanisms associated to these conditions.

Finally, it would also be interesting to explore Ng in other body fluids, such as plasma/serum. Despite in AD, plasma Ng levels are not altered compared to controls and do not display correlation with CSF Ng (De Vos et al., 2015), it is plausible that Ng alterations could be detected in the blood of CJD cases, where several surrogate markers of neuronal damage (e.g., tau, NFL) are increased compared to AD and controls.

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