BRAIN
SCIENTIFIC COMMENTARY

Peripheral tau as a biomarker for neurodegenerative diseases: is life on Earth, life on Mars?

This scientific commentary refers to 'Tau protein quantification in skin biopsies differentiates tauopathies from alpha-synucleinopathies' by Vacchi *et al.* (https://doi.org/10.1093/brain/awac161).

When searching for life on other planets, humans have historically based investigations on what we know about life on Earth—with its requirements such as water, oxygen and temperatures within a certain range. The problem though is that life on other planets may operate under different paradigms. The same principle may apply when evaluating peripheral biomarkers of neurodegenerative disease. Typically, researchers consider what defines a brain disease and then employ similar definitions and techniques to evaluate peripheral samples—even though composition, consistencies, methodologies and reproducibility may differ greatly between CNS and peripheral nervous system (PNS). This is no surprise as work needs to be hypothesis driven, with strong inference,[1] and thus it is logical to examine the *sine qua nons* of brain diseases, as Vacchi and co-workers[2] have done in this issue of *Brain*.

Neurodegenerative diseases are characterized by the progressive loss of selectively vulnerable populations of neurons.[3] The gold standard for diagnosis is the loss of those neurons and/or the presence of certain protein abnormalities in select neuroanatomical regions and cellular structures upon neuropathological evaluation after death.[3] For certain neurodegenerative diseases, including progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), the main aggregated protein within the brain is tau.

Tau is a microtubule-associated protein that exists as six major isoforms within the CNS, and specific post-translational modifications of tau, most notably phosphorylation, have been linked to neurodegenerative diseases.[4] A growing body of work has evaluated tau species in plasma, CSF and tissue biopsies. Studies examining tau species within tissues, such as skin, colon and submandibular gland, are dwarfed by those that have examined plasma and CSF. It is important to investigate peripheral tissues as these are more accessible than brain, may yield more consistent results due to their non-aqueous nature, and may provide a deeper understanding of the systemic nature of the neurodegenerative disorder, including the reasons why certain tissues are selectively vulnerable. Some studies, including our own[5,6] have investigated tau in human autopsy samples of peripheral tissues. Others have examined non-tau protein aggregates, such as alpha-synuclein, and have revealed good sensitivity and specificity of submandibular gland and skin biopsies for the diagnosis of Lewy type alpha-synucleinopathy and Parkinson's disease.[7,8]

Many biomarker studies, including that of Vacchi *et al.*[2] aim to provide a foundation for more sensitive and specific diagnoses of neurodegenerative diseases during life. To determine the sensitivity/specificity of biomarkers, one must first understand how a disease is defined, as well as the current diagnostic accuracy. One study reported 62% of persons clinical diagnosed as multiple system atrophy (MSA) could be confirmed at autopsy, while another found this figure to be as low as 53% for early Parkinson's disease.[9,10] Caution is thus needed when interpreting results of biomarker studies that use clinical diagnoses. Furthermore, biomarker studies must also consider generalizability and scalability. Much human research is conducted within a limited socio-economic/demographic distribution, hence the generalizability of findings should be evaluated. Scalability should also be discussed, including potential issues related to access, as well as costs.

Studies on peripheral biomarkers should involve specialist clinicians (e.g. behavioural neurologists or movement disorders specialists), biostatisticians, biochemists, molecular neuroscientists, as well as individuals with pathology expertise in the organ(s) examined, and neuropathology expertise at a minimum. This is necessary from an early stage to assure optimal study design, including a sample size sufficient to detect a meaningful effect, appropriate sampling parameters, evaluation methods and positive and negative controls. It will also help to ensure the influence of any confounding variables is recognized. This multidisciplinary evaluation should continue in the peer review process, and in any ensuing commentaries and editorials. Multi-stage tiered approaches are proposed for blood-based biomarkers; these can also be used for peripheral tissues.[11]

Vacchi *et al.*[2] evaluated levels of tau RNA and protein—including total and phosphorylated forms within skin (both cervical and leg) from clinically defined healthy controls and from patients with Parkinson's disease, MSA and PSP/CBD. The sampling parameter included a double 3 mm diameter punch skin biopsy on the more clinically affected side of the neck at C8 dermatomal level (cervical) and on the distal leg 10 cm above the lateral malleolus (ankle), with one sample fixed for immunofluorescence studies and the other stored for biochemical analyses. Using multiple techniques [immunofluorescence histochemistry, western blots, enzyme-linked immunosorbent assays (ELISA) and PCR] the authors revealed

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differences in tau abundance between diagnostic groups. The use of multiple methods, especially those which provide more quantitative results, such as ELISA, enhanced the scientific rigour of the study, while the use of commercially available reagents will make it easier to evaluate reproducibility in other research settings.

To investigate anatomical locations of select tau species, Vacchi *et al.*[2] performed immunofluorescence staining using antibodies against the N-terminal region (tau13), proline-rich domain (HT7 and Tau5) and tubulin binding region (Tau), revealing immunoreactive cutaneous nerve fibres counterstained with pan axonal markers or Beta tubulin class II (Fig. 1B in Vacchi *et al.*[2]). Tau-positive nerve fibres were also found in autonomic structures, and in epidermal and dermal regions where they co-localized with specific neurotransmitter markers (vasoactive intestine peptide and tyrosine hydroxylase) (Fig. 1C in Vacchi *et al.*[2]). Several phosphorylation-specific antibodies were used but no positive signals were detected.

Isoform expression studies revealed two major bands at 55 kDa (corresponding to the 0N4R/1N3R isoform of tau) and 70 kDa (corresponding to the 2N4R isoform). There was no evidence of 'Big Tau', the high molecular weight isoform identified in peripheral nerves. Using the same phosphorylation-specific antibodies, banding patterns were seen at 55 kDa and 70 kDa in western blot analyses with no significant differences found between groups. Cervical skin tau concentration measured by ELISA revealed increases in individuals with PSP/CBD when compared to healthy controls and individuals with Parkinson's disease, although there were large variations amongst participants (Fig. 4A in Vacchi *et al.*[2]). These measures were also related to age, further emphasizing the importance of evaluating other demographic metrics when developing biomarkers. Tau RNA results revealed the main transcript variants to be 2N and 4R with increases in 2N in both ankle and cervical skin in the PSP/CBD group, but all levels were minimal compared to that of the brain. This emphasizes the importance of using multiple methodologies and examining multiple forms of potential biomarkers (DNA, RNA, isoforms, post-translational modifications) when evaluating biomarkers, as different preparation methods can alter the results.

To summarize, Vacchi *et al.*[2] revealed greater abundance of specific tau species in the skin of individuals with clinically diagnosed PSP/CBD compared to healthy controls and to individuals with Parkinson's disease or MSA, using some methods but not all. Caution is required when interpreting the results given the limited number of participants in the study, the large number of comparisons and estimated correlations (although adjustment for multiple comparisons was conducted in some analyses), the lack of autopsy confirmation, which might affect sensitivity and specificity calculations, and the lack of an independent validation cohort for diagnostic performance (although leave-one-out validation was performed in the linear discriminant analysis). Given the small sample sizes, providing 95% confidence intervals with all reported results would have helped clarify the degree of variability in the findings. In addition, further work will be required to assess the reproducibility of techniques used and to explore how different laboratory parameters (fixation, section thickness, etc.) may affect results. Use of sequential western blots may introduce variability, for example, and may be limited as a diagnostic test due to the difficulty of scaling to a clinical setting. The authors discuss some of these limitations, especially the small cohort, lack of neuropathological confirmation, and discordances with previous studies, and are careful not to overstate findings.

Despite these limitations, this research is important as it adds to the body of work using multiple methodologies to evaluate the contributions of the PNS—revealed through examination of skin—to what central dogma states are CNS diseases. Future studies investigating the link between the periphery and brain in neurodegenerative disorders, should include expertise from multiple domains, understand the influence of pre-analytic factors, and have adequate power and effect size plus use of appropriate gold standards to validate specificity and sensitivity. To quote the 1964 classic 'Strong Inference' paper by Platt[1]: 'Devising a crucial experiment (or several of them) with alternative possible outcomes, each of which will, as nearly as possible exclude one or more of the hypotheses', can aid in our exploration of the question: is life on Earth, life on Mars?

Brittany N. Dugger,[1] Danielle Harvey,[2] Thomas G. Beach[3] and Charles H. Adler[4]

1 Department of Pathology and Laboratory Medicine, University of California Davis, Sacramento, CA 95817, USA
2 Division of Biostatistics, Department of Public Health Sciences, University of California, Davis, Davis, CA 95616, USA
3 Civin Laboratory for Neuropathology, Banner Sun Health Research Institute, Sun City, AZ 85351, USA
4 Department of Neurology, Mayo Clinic College of Medicine, Mayo Clinic Arizona, Scottsdale, AZ 85259, USA

Correspondence to: Brittany N. Dugger
E-mail: bndugger@ucdavis.edu

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Competing interests

The authors report no competing interests.

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