New biomarker could reveal Alzheimer's disease years before onset

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A study published today reported the identification of what may be the earliest known biomarker associated with the risk of developing Alzheimer's disease (AD). The results suggest that this novel potential biomarker is present in cerebral spinal fluid (CSF) at least a decade before signs of dementia manifest.

"If our initial findings can be replicated by other laboratories, the results will change the way we currently think about the causes of Alzheimer's disease," said Dr. Ramon Trullas, research professor at the CSIC Institute of Biomedical Research of Barcelona and lead author of the study that was published in *Annals of Neurology*. "This discovery may enable us to search for more effective treatments that can be administered during the preclinical stage."

Difficult Diagnosis

Alzheimer's disease affects more than five million Americans and is the sixth leading cause of death in the United States. At present, the only way to accurately diagnose the disease is by post-mortem neuropathological analysis. The relationship of currently known biomarkers with the cause of the disease is unclear, making it nearly impossible to diagnose preclinical stages of the disease with any real certainty.

The CSIC researchers demonstrated that a decrease in the content of

mitochondrial DNA (mtDNA) in CSF may be a preclinical indicator for Alzheimer's disease; furthermore, there may be a directly causative relationship. The hypothesis is that decreased mtDNA levels in CSF reflect the diminished ability of mitochondria to power the brain's neurons, triggering their death. The decrease in the concentration of mtDNA precedes the appearance of well-known biochemical Alzheimer's biomarkers (the A?1-42, t-tau, and p-tau proteins), suggesting that the pathophysiological process of Alzheimer's disease starts earlier than previously thought and that mtDNA depletion may be one of the earliest predictors for the disease.

In addition to enabling an investigation of the potential causal relationship of mtDNA and Alzheimer's progression, the use of mtDNA as an index of preclinical Alzheimer's disease provides an important advantage over previous biochemical markers: the detection of this novel nucleic acid biomarker is unhampered by the technical difficulties associated with protein detection. mtDNA can be readily quantified by real-time quantitative PCR (qPCR) or droplet digital PCR (ddPCR).

Quantitation of mtDNA

Prior to this study, researchers had not reported that circulating cell-free mtDNA could be detected in human CSF. But with this study, Dr. Trullas' team was able to both detect and reproducibly quantitate mtDNA using qPCR, carefully optimized by adhering to the MIQE guidelines.

To validate their qPCR findings, Dr. Trullas' team used Bio-Rad Laboratories' QX100™ Droplet Digital™ PCR system. Unlike qPCR assays, the QX100 system provides an absolute quantification of target DNA molecules without the need for a standard curve. In addition, an important factor for their CSF analysis was that the Droplet Digital PCR system did not require sample purification to remove PCR inhibitors, as

is necessary for qPCR assays.

"Droplet Digital PCR allowed us to validate our initial qPCR measurements because it provides absolute quantitation at the single-molecule level without relying on a standard curve," said Dr. Trullas. "As the technology becomes more widely adopted, we anticipate that Droplet Digital PCR will be the future of detecting mtDNA in cerebral spinal fluid."

Dr. Trullas hopes that other laboratories and hospitals will successfully replicate his group's research results, confirming that reduced mtDNA levels should be investigated as a possible cause of Alzheimer's disease. By finding a way to block this degeneration, clinicians may be able to diagnose and treat Alzheimer's disease before symptoms appear.

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