Identification of biofluid markers of TDP-43 pathology

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INTRODUCTION

Frontotemporal dementia (FTD) is a neurodegenerative disorder usually presenting either with a change in personality (behavioural variant FTD) or language impairment (primary progressive aphasia), although around 20% of patients also have features of motor neurone disease (known as FTD-MND) (Woollacott et al, 2016). The discovery in 2006 of TAR DNA binding protein 43 kDA (TDP-43) as the main component of pathological inclusions in cases of FTD provided a major molecular insight into the disease. It has since become clear that the TDP-43opathies are the most common pathological form of FTD accounting for around 60% of cases, with the tauopathies accounting for most of the rest. However, it is still not possible during life to distinguish which pathological group an individual patient with FTD falls into (except for those with genetic mutations), because of poor clinico-pathological correlation.

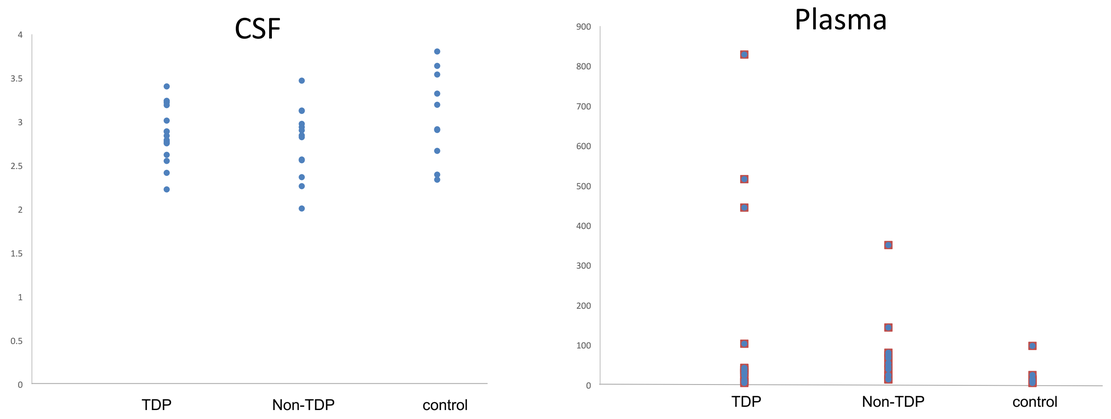
Widely available assays for CSF TDP-43 seem to represent TDP-43 levels in blood and are not a measure of central nervous system TDP-43 pathology (Feneberg et al, 2014). They are therefore unable to distinguish between the TDP-43opathies and the tauopathies in patients with FTD.

METHODS

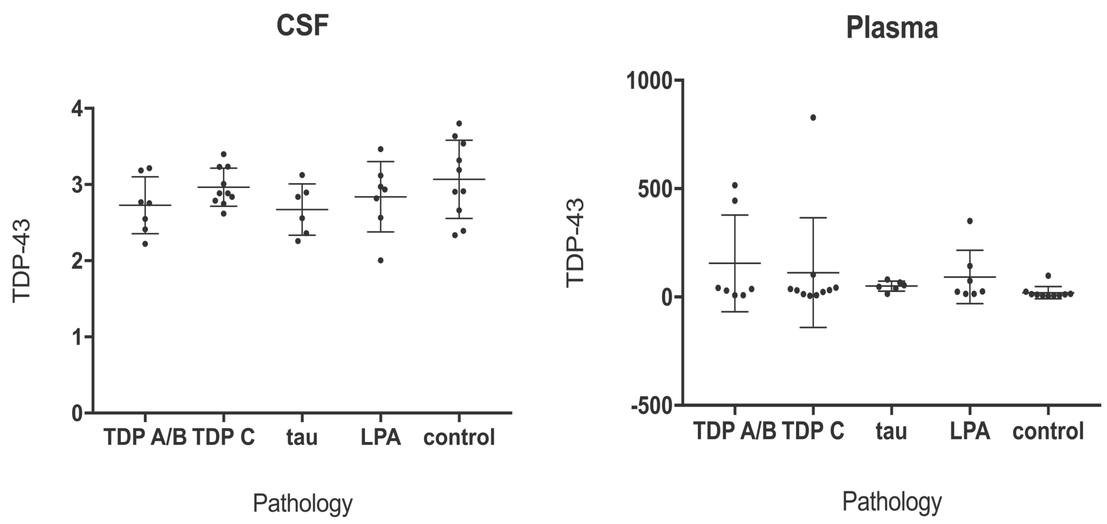
The novel TDP-43 Simoa assay (Quanterix) has been developed with antibodies against amino acids 203-209 and the C-terminal region. We set out to investigate this assay in both plasma and CSF in a cohort of patients recruited from the University College London FTD observational studies with known or likely TDP-43 pathology (7 cases with TDP type A or B, 10 with TDP type C), non-TDP-43 pathology (6 with likely tau, and 7 with likely Alzheimer's disease pathology) and healthy controls (10).

EDTA plasma and CSF samples were collected from the participants, processed and stored at −80°C following standardised procedures. Plasma level concentrations were measured using the Human TDP-43 kit (Quanterix, Boston, Massachusetts, USA) with the Simoa HD-1 Analyser (Quanterix, Boston, Massachusetts, USA).

RESULTS



The mean [standard deviation] plasma TDP-43 concentration was higher in those with likely TDP-43 pathology (155.1 [223.4] pg/ml) than those with non-TDP pathology (112.39 [252.9] pg/ml), and healthy controls (50.0 [23.1] pg/ml), but the differences between groups was non-significant, with substantial overlap in concentrations between all three groups. Interestingly, 4 patients had very high TDP levels, the significance of which is unclear. The mean CSF TDP-43 concentration was 2.9 [0.3] pg/ml in those with likely TDP-43 pathology, 2.8 [0.4] pg/ml in those with non-TDP pathology, and 3.1 [0.5] pg/ml in healthy controls. There remained no significant difference when TDP-43 levels were compared across the pathological subtypes.



HYPOTHESIS

The main hypothesis is that TDP-43 pathology can be reliably distinguished from other pathological forms of FTD using novel ultrasensitive assays, allowing accurate molecular diagnosis in life.

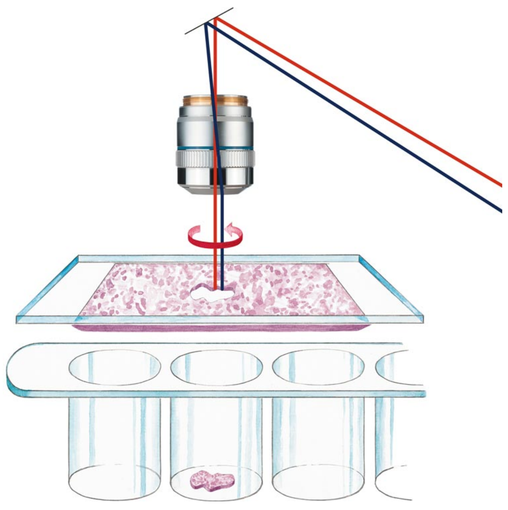
AIM

My overarching goal is to develop an assay that will detect specifically the abnormal isoforms of TDP-43 implicated in disease. The project will use a state-of-the-art Simoa HD-1 Analyzer (Quanterix), which is capable of single molecule detection and has a sensitivity up to 1000-fold greater (subfemtomolar range) than current ELISA approaches. The three key aims are:

1.To identify specific proteins and protein fragments associated with the TDP-43opathies, using laser capture microdissection coupled with mass spectrometry.
2.To use the findings from aim 1 to develop novel ultrasensitive assays for pathology-specific/enriched TDP-43 species and associated proteins using Simoa technology.
3.To test the assays developed in aim 2 in a deeply phenotyped cohort of patients with FTD including examination of whether concentrations correlate with measures of disease severity and/or progression

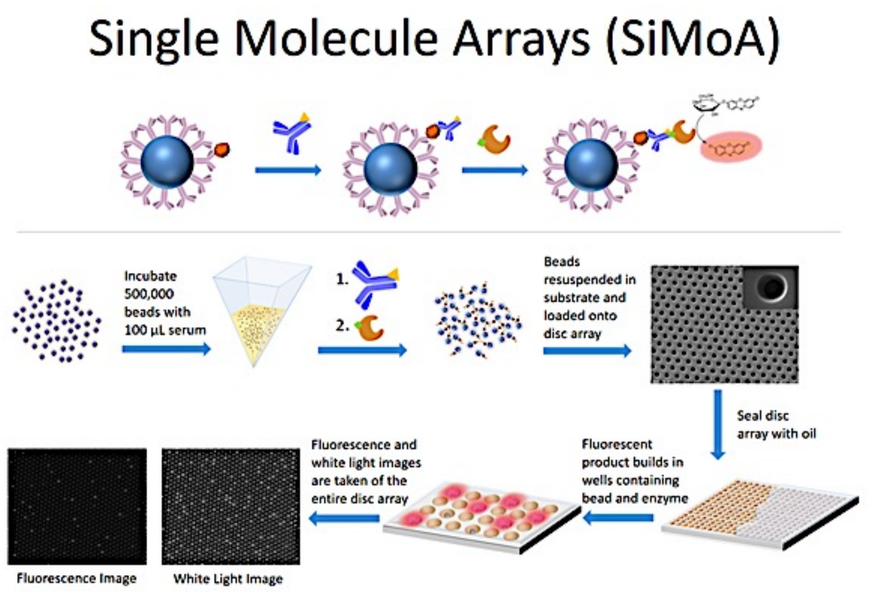
EXPERIMENTAL PLAN

Laser capture microdissection
Available tissue from patients who have donated their brains to the Queen Square Brain Bank will be used as the starting point of the project. Frozen brain sections (10µm) will be mounted onto PEN membrane slides (Leica) for use with the Leica laser capture micro-dissection microscope (LMD 7000). Sections will undergo rapid immunohistochemistry to identify the TDP-43 inclusions whilst preserving the protein integrity in the tissue.



Mass spectroscopy
Using laser-captured TDP-43 inclusions, mass spectrometric analysis (high-resolution nanoLC-MS/MS quantitative proteomics) will identify and quantify TDP-43 species and associated proteins

Simoa assay development
Monoclonal antibodies against inclusion-specific or -enriched TDP-43 isoforms will be made with selection of pairs for which at least one of the antibodies has neoepitope- or isoform-specificity and works well in regular ELISA. The most promising ELISAs will be transferred onto the Simoa HD-1 Analyzer.



Test of new assay on a deeply phenotyped cohort of patients with FTD
Biosamples for patients with FTD have been collected as part of two major studies at UCL, the Genetic FTD Initiative (GENFI) which is a multicentre study of genetic FTD run from UCL, and the Longitudinal Investigation of FTD (LIFTD) study of sporadic FTD and associated disorders. All participants have been deeply phenotyped with available clinical, neuropsychological and neuroimaging data allowing correlation of bioassay concentrations with measures of disease severity and progression.

References: Woollacott, I. O. C., & Rohrer, J. D. (2016). The clinical spectrum of sporadic and familial forms of frontotemporal dementia. *Journal of Neurochemistry*, *138*(S1), 6–31. Feneberg, E., Steinacker, P., Lehnert, S., Schneider, A., Walther, P., Thal, D. R., … Otto, M. (2014). Limited role of free TDP-43 as a diagnostic tool in neurodegenerative diseases. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, *15*(5–6), 351–356.
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