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, 2014). The discovery in 2006 of TAR DNA binding protein 43 (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 levels are not a measure of central nervous system TDP-43 levels. They are therefore unable to distinguish between the TDP-43opathies and the tauopathies in patients with FTD, either with a change in personality (behavioural variant FTD) or language impairment. This is supported by an Alzheimer’s Research UK Clinical Research Training Fellowship (ARUK Clinical Neuroscience, UK) and a University College London FTD observational study of known or likely TDP-43 pathology, which involves examination of whether concentrations correlate with measures of disease severity and progression.

METHODS

The novel TDP-43 Simoa assay (Quanterix) has been developed with antibodies against amino acids 203-208 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 (151.2 [223.4] pg/ml) than those with non-TDP-43 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-43 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 ultra-sensitive 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 ultra-sensitive 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 aepitope- or isoform-specificity and works well in regular ELISA. The most promising ELISAs will be transferred onto the Simoa HD-1 Analyzer.

REFERENCES


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