IN VITRO AGGREGATION ASSAY OF THE TRANSACTIVE RESPONSE (TAR) DNA-BINDING PROTIEN OF 43-kDa (TDP-43).

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INTRODUCTION

Aggregated forms of the transactive response (TAR) DNA-binding protein of 43 kDa (TDP-43) are the major neuropathological hallmark in the central nervous system (CNS) of patients with Frontotemporal Lobar Degeneration (FTLD-TDP) and Amyotrophic Lateral Sclerosis (ALS), also defined as TDP-43-proteinopathies (1). Aggregated pathological TDP-43 is ubiquitinated, phosphorylated and proteolytically cleaved into C–terminal fragments. Several studies have demonstrated that TDP-43 is able to misfold and transmit its abnormal conformation in a prion-like manner (2). An important assay, named Real Time Quaking Induced Conversion (RT-QuIC), has been developed in the prion field with the aim of reproducing the misfolding process *in vitro*. RT-QuIC is an ultrasensitive assay, able to detect trace-amount of pathological protein present in different tissues (blood, urine, CSF) used as supporting diagnostic tool for prion diseases (3).

OBJECTIVES

The aims of this work were to detect TDP-43 pathological aggregates in the brain homogenates of patients with FTLD and optimize the RT-QuIC conditions for the aggregation of recombinant TDP-43.

MATERIALS AND METHODS

Soluble or insoluble fractions (collected after sarkosyl treatment) of TDP-43 were obtained from brain homogenates of patients with FTLD. The same fractions were collected from control brains. Immunohistochemistry was performed on brain tissues using a monoclonal anti-phospho-TDP-43 antibody (Ser409/Ser410). The presence of pathological TDP-43 in both fractions was assessed by means of Western blot. RT-QuIC preliminary experiments were performed using the full length recombinant TDP-43 protein (recTDP-43^{FL}) at the concentration of 5 μg/100 μL. Reaction was performed alternating 1 minute of shaking to 1 minute of incubation at 37°C.

RESULTS

Western blot analysis for TDP-43 of the insoluble fractions demonstrated two positive samples among the diseased cohort with bands migrating at ~25 kDa. Two other patients demonstrated the presence of these bands with less intensity **(Figure 1a).** The presence of pathological TDP-43 was demonstrated only in the insoluble fraction of FTLD patients while was not found in the soluble one (**Figure 1b**). The pathological protein (i.e. bands at ~25 kDa) was not found in controls samples. The two positive patients demonstrated a different banding pattern that was replicated in different blot analysis. Immunohistochemistry with anti-pS409/410 monoclonal antibody confirmed the presence of TDP-43 pathological aggregates in diseased brains which resulted positive at Western blot analysis **(Figure 2**). The RT-QuIC conditions for the aggregation of recTDP-43^{FL} including the concentration of recombinant protein and temperature were partially optimized. The recTDP-43^{FL} showed a very fast aggregation kinetics (**Figure 3**) and further modifications in the experimental setting are required to slow it down (e.g. temperature, time of incubation/shaking).

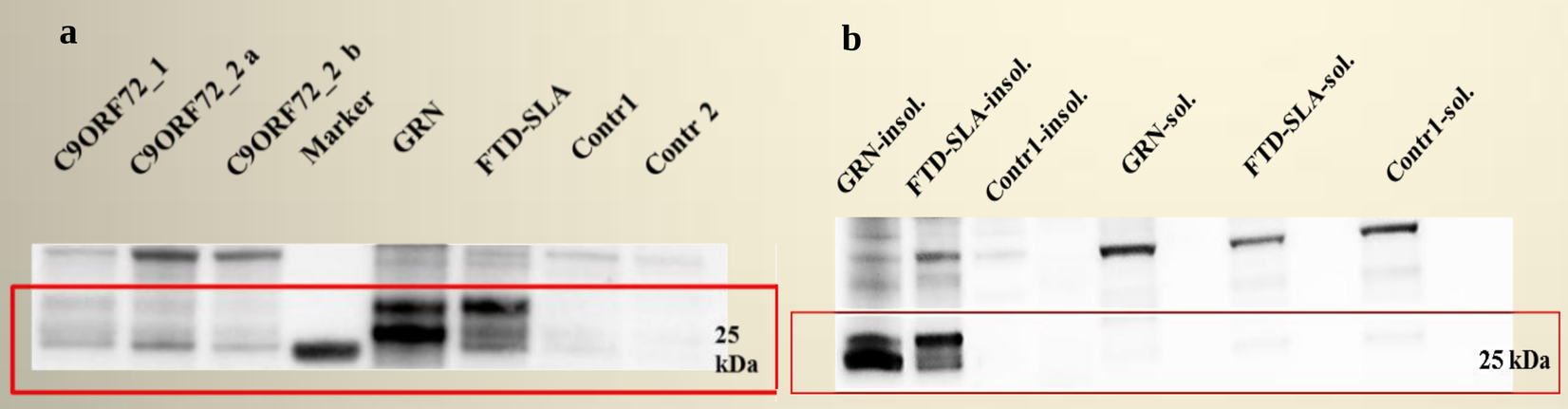

Figure 1. a) Western blot analysis performed with anti-pS409/410 monoclonal antibody detected the presence of TDP-43 pathological bands at ~25 kDa in two subjects (GRN and FTD-SLA) and not in controls; two other subjects (C9ORF72_1 and C9ORF72_2) resulted positive but with a lower intensity of the signal; b) the same analysis was performed on the soluble and insoluble fractions and revealed that pathological bands were identified only in the insoluble one.

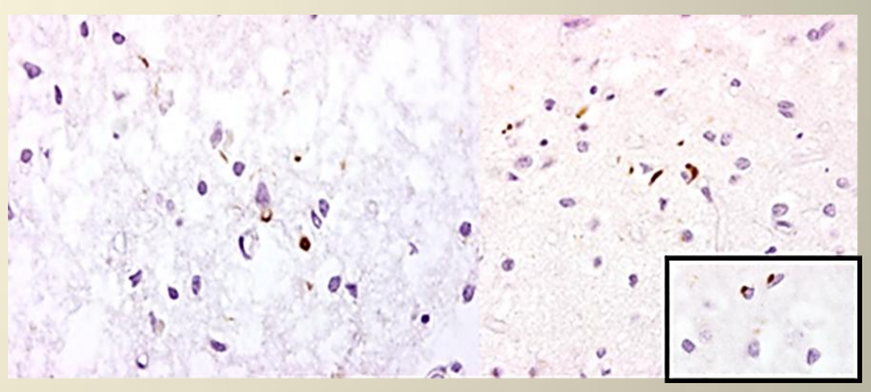

Figure 2. Immunohistochemistry with anti-pS409/410 monoclonal antibody confirmed the presence of intracytoplasmic TDP-43 inclusions associated with dystrophic neurites.

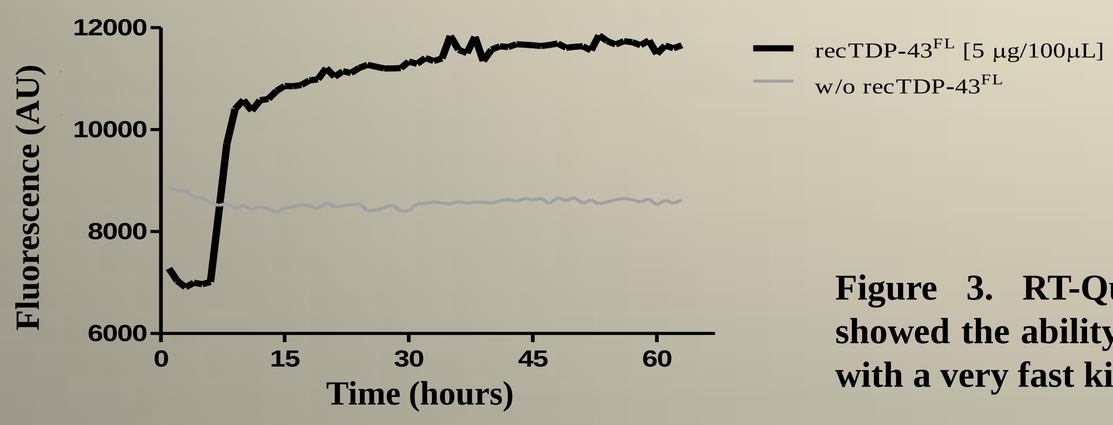

Figure 3. RT-QuIC reaction of recTDP-43^{FL} showed the ability of the protein to self-aggregate with a very fast kinetic (black line).

SUMMARY

✓WE WERE ABLE TO EXTRACT PATHOLOGICAL TDP-43 FROM DISEASED BRAIN CONFIRMING ITS PRESENCE BY MEANS OF WESTERN BLOT ANALYSIS

✓WE SET UP THE FIRST RT-QUIC PROTOCOL TO INDUCE recTDP-43^{FL} TO AGGREGATE (WITH THE AIM OF DETECTING TRACE-AMOUNT OF PATHOLOGICAL TDP-43)

✓WE OBSERVED A VERY FAST AGGREGATION KINETIC OF TDP-43 BY MEANS OF RT-QUIC

✓ PATHOLOGICAL TDP-43 IN PATIENTS COULD BE ALSO PRESENT AND, THUS, DETECTED IN PERIPHERAL TISSUES

✓FURTHER EXPERIMENTS ARE NEEDED TO OPTIMIZE THIS TECHNIQUE AND USE IT AS SUPPORTING DIAGNOSTIC TOOL FOR TDP-43 PROTHEINOPATIES (USING PERIPHERAL TISSUES OF DISEASED PATIENTS).

DISCUSSION

Our results confirm the ability of TDP-43 to self-aggregate and encourage further research in order to define the most appropriate setting for the use of the RT-QuIC reaction as supporting tool in the diagnosis of TDP-43-proteinopaties. In fact, once optimized, this technique could detect small quantities of pathological protein in a given sample, overcoming the limits of quantitative assays that, so far, failed in discriminating between patients and controls when using TDP-43 as a peripheral biomarker.

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