RAPID ASSAYS OF CUTANEOUS PROTEIN BIOMARKERS FOR PARKINSON'S DISEASE

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Abstract: Parkinson's disease (PD) is a common neurodegenerative disease that is characterized by the deposition of misfolded protein aggregates such as α-synuclein in the central nervous system (CNS). There remains an urgent need for reliable, inexpensive and minimally invasive assays for peripheral biomarkers. Recent studies have revealed widespread peripheral involvement of PD-like pathology, often prior to clinical manifestations of the disease. Indeed, α-synuclein deposits have been observed in peripheral tissues in PD. A formidable challenge is that the levels of these protein aggregates in peripheral tissues are extremely low and thus only variably detectable using immunological methods. Therefore, highly sensitive analytical platforms are requiredfor biomarker assays specific for protein aggregates. The real-time quaking induced conversion (RT-QuIC) has emerged as a robust, rapid and ultrasensitive technology for template-assisted amplification of misfolded protein aggregates in neurodegenerative diseases. Using the RT-QuIC technique, our recent studies have demonstrated rapid and ultrasenesitive detection of α-synuclein aggregates in peripheral skin tissue of patients affected by PD. Reliable assays for peripheral protein biomarkers will enable sensitive premortem diagnostic tests for neurological diseases such as PD.

Keywords - Biomarker,Parkinson's Disease, α-Synuclein.

I. INTRODUCTION

Parkinson disease (PD) is the second most common neurodegenerative disease, affecting about 1 million people in the U.S. The cardinal neuropathological hallmark of PD is the accumulation of the disease-associated α-synuclein (□Syn^{D}) aggregates in the form of Lewy bodies or Lewy neurites in the brain (Spillantini et al. 1998, Braak et al. 1999). Currently, a definitive diagnosis relies on detection of the □Syn^{D}-containing neuronal inclusions in autopsied brain tissues (Dickson et al. 2009). To date, there has been no reliable test for PD, i.e., a biomarker that can be used for diagnosis, monitoring disease severity, or evaluating therapeutic efficacy (Chen-Plotkin et al. 2018). Thus, identifying reliable biomarkers for PD is highly significant.

Previous efforts have focused on the development of CNS-proximal clinical biomarkers, including PET neuroimaging and cerebrospinal fluid measurements of total or phosphorylated □Syn. However, these diagnostic techniques are often used in clinical studies on patients with advanced disease state, and are complex, invasive or expensive.

The real-time quaking induced conversion (RT-QuIC) is an ultrasensitive assay based on the ability of misfolded protein aggregates to induce, or "seed", the polymerization of monomeric protein into amyloid fibrils (Atarashi et al. 2011, Orru et al. 2017) The RT-QuIC reaction can result in many orders of magnitude signal amplification of original seeds due to an exponential increase of amyloid fibrils that enhance the fluorescence of the dye thioflavin T (ThT). The RT-QuIC assay was initially developedto detect prion protein aggregates and for the diagnosis of Creutzfedt–Jakob disease (CJD)(Atarashi et al. 2011, Orru et al. 2017).Recently, RT-QuIC assay has been adapted todetect□Syn^{D}in the cerebrospinal fluid (CSF) of PD patients(Fairfoul et al. 2016, Shahnawaz et al. 2017, Groveman et al. 2018). However, lumbar puncture to obtain CSF specimens is not typically included in the diagnostic process outside of clinical studies due to the invasive CSF collection process that limits its application. Ideally, RT-QuIC assay of □Syn^{D}in easily accessible peripheral tissue would enable premortem and even preclinical diagnosis of PD.

Using immunohistochemical (IHC) methods, the □Syn immunoreactive, Lewy-like□deposits have been previously observed throughout the peripheral nervous system innervating organs such as colon, salivary gland, and skin (Braak et al. 2006, Beach et al. 2010), and have been postulated to precede the brain pathology and contribute to autonomic dysfunction in PD (Braak et al. 2003, Braak and Del Tredici 2017). DetailedIHC studies had led to the identification of skin □Syn deposits within autonomic and sympathetic nerve endings of PD patients (Donadio et al. 2014, Doppler et al. 2014, Zange et al. 2015, Gibbons et al. 2016, Donadio et al. 2017). Because autonomic dysfunction associated with skin nerve abnormality may appear long before clinical symptoms in PD patients and individuals at-risk (Gibbons et al. 2016, Antelmi et al. 2017), cutaneous □Syn^{D} is likely a good candidate for monitoring disease progression. However, IHC of skin □Syn has been historically challenging as widely inconsistent low sensitivity from 0-100% were

obtained due to inherent high variability of the IHC procedures (antibody specificity, tissue fixation, antigen retrieval, and tissue sectioning/embedding (Lee et al. 2017, Visanji et al. 2017).

We have applied RT-QuIC platform for rapid and ultrasensitive detection of □Syn^{D} in skin specimens of PD patients using a commercial fluorescence plate reader.

II. EXPERIMENTAL DETAILS

2.1. RT-QuIC Platform

In the current format, samples containing αSyn^{D} are incubated with excess amounts of recombinant □Syn in the presence of ThT in a 96-well plate within a fully automated commercial shaker-plate reader (Fig. 1). The kinetic plots of increased ThT fluorescence over time reflect the amplified detection of αSyn^{D} in a given sample.

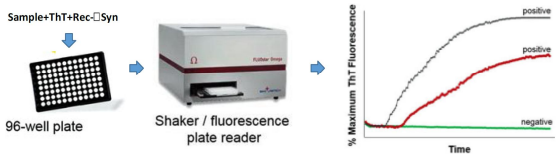


Fig. 1. Diagram of RT-QuIC assay for aSyn^{D}

2.2. Experimental Procedures

Approximately 30 mg of skin tissuewas homogenized in TBS containing 2 mM CaCl2 and 0.25% (w/v) collagenase A (Roche) as described previously (Orrú et al., 2017). The mixture was centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was serially diluted. RT-QuIC reaction mixture was composed of 10 mM PBS (pH 7.4), 300 mM NaCl, 0.1 mg/ml recombinant human αSyn, and 10 µM ThT. Aliquots of the reaction mixture (98 µl) was loaded into each well of a black 96-well plate containing 6 zirconium/silica beads per well.Skin homogenate (2 µl) at about 10^{-3}-10^{-5} dilutions was added. The plate was sealed plate and incubated at 42°C in a Biotek SynergyH1 plate reader with cycles of 1 min shaking (425 rpm double orbital) and 1 min rest throughout the indicated incubation time (up to 72 h).

III. RESULTS AND DISCUSSION

3.1. Rapid and Sensitive Detection of □Syn^{D}in Skin Specimens of PD PatientsUsing RT-QuIC Assay

In the present study, we tested 3 frozen autopsy skin specimens from abdominal area of either PD patients or non-PD control subjects. The RT-QuIC assay readily detected aSyn^{D} in PD skin homogenatesbut not in non-PD controls ata dilution of 10^{-5}in 60 h (Fig. 2). The high sensitivity and specificity of skin RT-QuIC assay for PD as demonstrated in this proof-of-principle pilot study willmotivate further validation in large cohorts of PD patients.

Cutaneous aSyn^{D} RT-QuIC assayhas the potential to serve as a useful biomarker for PD, to enable premortem and preclinical diagnosis, monitoring disease onset and progression, and assessment of efficacies for future therapeutics.

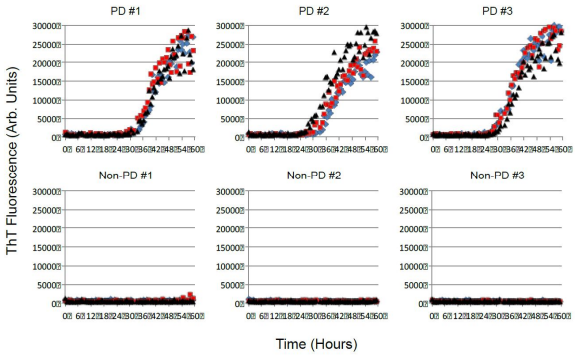


Fig. 2. RT-QuIC analysis of aSyn^{D}

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