Abstract

Prionopathies, also called transmissible spongiform encephalopathies (TSE) and synucleinopathies are neurodegenerative diseases that are associated with the accumulation of misfolded proteins (prion and α-synuclein) mostly in the central nervous system. To this day, early and definite diagnosis remains unavailable during the patient's lifetime, mainly due to the absence of reliable biomarker which makes clinical diagnosis more challenging. Therefore, the gold standard in diagnostics remains direct *post-mortem* evaluation of misfolded proteins within brain tissue by western blot and immunohistochemistry. In the recent years, seeding amplification assays (SAAs) like Real-Time Quacking-Induced Conversion (RT-QuIC) emerged for ultra-sensitive *ante-mortem* diagnosis of neurodegenerative diseases. SAAs exploit ability of pathological misfolded proteins present in patient's samples to change the conformation and initiate aggregation of native recombinant protein substrate by prion-like seeding mechanism.

In the presented dissertation thesis, we exploited second-generation RT-QuIC assay (55°C, 700 rpm, cycles of 1 min double-orbital shaking and 1 min incubation) utilizing recombinant hamster shortened prion protein (rHAPrP90-231) to evaluate prion seeding activity in *post-mortem* TSE (n=38) and non-TSE (n=30) cerebrospinal fluid (CSF) and corresponding skin samples. In CSF, we were able to achieve 100% sensitivity and specificity after dilution of samples to remove the effect of present inhibitors. In the skin samples, the sensitivity and specificity of the assay was 89.5% and 100%, respectively. Interestingly, the analysis showed higher median prion seeding dose in the skin samples than in corresponding CSF. To further explore diagnostic potential of skin, we analyzed skin (head apex and ear lobe) from mice inoculated intracerebrally or subcutaneously with RML prion strain. Subcutaneously infected mice showed positive RT-QuIC results in the skin from the apex and ear lobe 12 days and 40 days before the onset of symptoms, correspondingly. However, intracerebrally infected mice displayed positive prion seeding activity in skin only after the onset of symptoms. Moreover, we examined prion seeding activity in two siblings with a genetic Creutzfeldt-Jakob disease with a novel five octapeptide repeats insertion (5-OPRI, R1-R2-R2-R3-R4-**R2-R2-R3-R3-R4**) in the *PRNP* gene. Duration of the disease was more than 10 years in both patients. In the first case, positive seeding activity was detected in every type of tested sample (frontal lobe, cerebellum, CSF, skin). On the contrary, in the second case, no RT-QuIC positivity was detectable suggesting the possible presence of different prion strain. We successfully adopted a protocol for RT-QuIC analysis of formalin-fixed paraffin-embedded brain tissue of TSE patients and demonstrated its ability to detect prions in cohorts of TSE (n=30) and control non-TSE (n=30) patients.

Furthermore, we established RT-QuIC assay adapted for synucleinopathies (42°C, 400 rpm, cycles of 1 min double-orbital shaking and 1 min incubation). We validated α-syn seeding activity in 15 *post-mortem* brain homogenates and CSF samples with definite Dementia Lewy bodies (DLB, n=6), Alzheimer disease with Amygdala Lewy bodies (AD/ALB, n=3) and Creutzfeldt-Jakob disease with DLB (CJD/DLB) comorbidity (n=6) with 100% and 92.9% sensitivity, respectively. We also reported detection of α-syn higher seeding activity in a few non-TSE control samples. However, in two of them, secondary synucleinopathy was confirmed after the neuropathological reevaluation which was prompted by our RT-QuIC results.

Key words: diagnosis, neurodegenerative diseases, prion, CJD, α-synuclein, synucleinopathy, RT-QuIC