Sporadic Creutzfeldt-Jakob disease: Real-Time Quaking-Induced Conversion (RT-QuIC) assay represents a major diagnostic advance

Federico Angelo Cazzaniga*, Edoardo Bistaffa*, Chiara Maria Giulia De Luca, Antonio Indaco, Giuseppe Bufano, Giorgio Giaccone, Fabio Moda

Fondazione IRCCS Istituto Neurologico Carlo Besta, Division of Neurology 5–Neuropathology, Milan, Italy

*These authors equally contributed to this work

Sporadic Creutzfeldt-Jakob disease (sCJD) is a rare and fatal neurodegenerative disorder with an incidence of 1.5 to 2 cases per million population/year. The disease is caused by a proteinaceous infectious agent, named prion (or PrPSc), which arises from the conformational conversion of the cellular prion protein (PrPC). Once formed, PrPSc interacts with the normally folded PrPC coercing it to undergo similar structural rearrangement. The disease is highly heterogeneous from a clinical and neuropathological point of view. The origin of this variability lies in the aberrant structures acquired by PrPSc. At least six different sCJD phenotypes have been described and each of them is thought to be caused by a peculiar PrPSc strain. Definitive sCJD diagnosis requires brain analysis with the aim of identifying intracerebral accumulation of PrPSc which currently represents the only reliable biomarker of the disease. Clinical diagnosis of sCJD is very challenging and is based on the combination of several clinical, instrumental and laboratory tests representing surrogate disease biomarkers. Thanks to the advent of the ultrasensitive Real-Time Quaking-Induced Conversion (RT-QuIC) assay, PrPSc was found in several peripheral tissues of sCJD patients, sometimes even before the clinical onset of the disease. This discovery represents an important step forward for the clinical diagnosis of sCJD. In this manuscript, we present an overview of the current applications and future perspectives of RT-QuIC in the field of sCJD diagnosis.

Key words: Sporadic Creutzfeldt-Jakob disease; olfactory mucosa; cerebrospinal fluid; neurodegeneration; peripheral biomarkers; prion; seeding aggregation assays.

Correspondence: Giorgio Giaccone, Fondazione IRCCS Istituto Neurologico Carlo Besta, Division of Neurology 5–Neuropathology, Via G. Celoria 11, 20133 Milan, Italy. E-mail: giorgio.giaccone@istituto-besta.it

Contributions: FAC, EB, manuscript drafting, tables and figures preparation; CMGD, AI, GB, contribution to manuscript drafting; FM, GG, manuscript concept and critical revision. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: The authors declare that they have no competing interests, and all authors confirm accuracy.
Molecular and neuropathological classification of sCJD subtypes

Among human prion diseases, sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form affecting 1-2 individuals/million per year with similar distribution in males and females. The age at onset is most frequently between 55 and 75 years. sCJD presents with variable disease subtypes characterized by peculiar clinical and neuropathological features. In the past, other than the classical and more common subtypes, some clinical variants such as the Heidenhain, the myoclonic, the thalamic, the cerebellar or ataxic, and the panencephalopathic forms were reported. In general, sCJD cases present as multifocal and rapidly progressive encephalopathies with dementia, cerebellar ataxia, myoclonus while the progression of the disease results in an akinetic and mute state and the death occurs generally within 6 months after the disease onset. The common mechanism underlying these pathologies is the spontaneous conformational conversion of the cellular prion protein (PrPC) into an abnormally folded conformer named prion or PrPSc. This latter propagates in an autocatalytic manner in the brain by converting the PrPC into the pathological isoform.

PrPC is a glycosylphosphatidylinositol (GPI) anchored protein highly expressed in the central nervous system (CNS) and encoded by the PRNP gene located on chromosome 20 in humans. After its synthesis in the rough endoplasmic reticulum, PrPc undergoes post-translational modifications comprising the C-terminal addition of the GPI anchor, the formation of a disulfide bridge between two C-terminal cysteine residues (Cys179-Cys214) and the N-linked glycosylation at asparagine residues (Asn181-Asn197). These oligosaccharides are further modified in the Golgi apparatus to produce complex-type chains enriched in sialic acid important for the synaptic localization of PrPC. The different degrees of PrPc glycosylation give rise to three isoforms of the protein: the di-glycosylated (70%), the mono-glycosylated (25%) and the un-glycosylated (5%) species. All these isoforms are rich in α-helices structures, soluble in detergent and are sensitive to proteolytic digestion with proteinase K (PK). Conversely, PrPSc is less soluble in detergent, has higher amount of β-sheet structures and is partially resistant to PK digestion. The limited proteolysis leads to the generation of N-terminal truncated fragments of di-, mono- and un-glycosylated PrPSc that migrate at lower molecular weights compared to those of PrPc. Moreover, the un-glycosylated band of PrPSc can acquire two distinct molecular weights: 21 or 19 kDa which are referred to as type 1 or type 2 PrPSc, respectively. Neuropathologically, the main hallmarks of sCJD are spongiform changes, astrogliosis and accumulation of PrPSc (Figure 1 and Figure 2).

At present, PrPSc is the only disease-specific biomarker for sCJD and the definite diagnosis can be formulated post-mortem by biochemical and neuropathological analyses aimed at identifying the PrPSc accumulation in the CNS (Figure 2).

It is well known that PrPSc can acquire different abnormal conformations, named strains. The peculiar conformation of each strain can be faithfully transmitted to the host PrPc and are believed to be responsible for the heterogeneity of prion diseases, in terms of tissue tropism, incubation period, clinical signs, neuropathological changes and interspecies transmission properties. In 1999, Parchi and colleagues classified sCJD in six major subtypes by correlating the clinical manifestations with the polymorphisms at codon 129 of the PRNP gene, i.e. methionine (M) or alanine (A).

Figure 1. Creutzfeldt-Jakob disease, hallmark neuropathologic lesions. Spongiform changes may appear as small vacuoles (A) diffusely present in grey matter (H&E, cerebral cortex, 20x) or large, confluent vacuolar lesions (B) typical of the MM2-C (cortical) subtype (H&E, cerebral cortex, 20x). Kuru plaques (C) small aggregates of PrP with the tinctorial and optical properties of amyloid are typically found in the cerebellum in MV2 subtype (H&E, 60x). Astrogliosis (D) may be severe in all subtypes of Creutzfeldt-Jakob disease (glial fibrillary acidic protein immunohistochemistry, 10x). Neuronal loss (E) is usually very severe in the cerebral cortex, basal ganglia and cerebellum (H&E, cerebellum) but may be mild in some cases (F) (microtubule associated protein 2 immunohistochemistry; 10x).
valine (V), and the electrophoretic mobility of the un-glycosylated PrP<sub>S</sub> isoform in the brain after digestion with PK (type 1 or type 2 PrP<sub>S</sub>). These findings demonstrated that the presence of M or V at codon 129 of PrPC, as well as other still unknown factors, could modulate the structural rearrangement of PrP<sub>D</sub> during misfolding, thus promoting the PrP<sub>S</sub> strains variability. In addition, compelling evidence suggests that, in some sCJD cases, the CNS contains a mixture of PrP<sub>Sc</sub> strains (e.g., MM1+2, VV1+2 and MV1+2), which make the classification of the disease even more challenging (as discussed in the next paragraphs).

The main pathological characteristics of each sCJD subtype are summarized in Table 1.

**MM1 and MV1 subtype**
MM1 is the most common form of sCJD (67% of all cases) while MV1 cases are rare (3%). Western blot analysis shows, for both subtypes, type 1 PrP<sub>S</sub> and a glycoform pattern characterized by the predominance of the mono-glycosylated band. Despite the difference at codon 129 of PRNP, MM1 and MV1 cases share many pathological features. MM1/MV1 CJD patients present with the myoclonic (or classic CJD) and the Heidenhain’s variant. The mean age at onset of the disease is 66 years with an average clinical duration of 4 months. Clinical manifestations include cognitive impairment with memory loss and confusion/disorientation, depression, anxiety, psychosis and gait or limb ataxia.

Neuropathologically, the brain of these patients shows spongiosis with fine vacuoles. The basal ganglia, thalamus and cerebellum are less affected than the cerebral neocortex. The hippocampal cortex and brain stem are largely spared. The pattern of PrP<sub>S</sub> deposition is synaptic and mainly affects the cerebral cortex while the cerebellum, the basal ganglia and thalamus are less involved (Figure 2 A,B). Moreover, the amount of PrP<sub>S</sub> signal directly correlates with the severity of spongiosis.

**VV2 subtype**
The VV2 subtype corresponds to the cerebellar or ataxic variant and occurs in 15% of sCJD cases. The Western blot profile shows type 2 PrP<sub>S</sub> with a preponderance of the mono-glycosylated isoform. The mean age at onset is 64 years (with a range of 40-83 years) and the clinical duration is about 7 months. Ataxia is the commonest early clinical feature accompanied by cognitive impairment and oculomotor signs while myoclonus is less frequent. In the late stages of the disease patients exhibit dementia, myoclonus and pyramidal signs. Neuropathologically, the spongiosis preferentially affects the deep layers of the frontal and occipital cortex, the entorhinal cortex and the hippocampus. Cerebral neocortex may be relatively spared particularly in cases with rapid courses. The cerebral cortex is atrophic, with abundant PrP<sub>S</sub> deposits characterized by a focal and plaque-like pattern that are negative for Congo Red and Thioflavin-S (amyloid stains). In addition, strong PrP<sub>S</sub> deposition often occurs around neuronal perikarya in the cerebral cortex (Figure 2 C,D). The distribution of PrP<sub>S</sub> immunostaining is affected by the disease duration. In cases with shorter disease duration, PrP<sub>S</sub> involve diffusely the gray-matter region except for the neocortex which is affected only in patients with longer disease duration.

**MV2 subtype**
MV2 sCJD subtype is phenotypically and biochemically similar to VV2 cases (type 2 PrP<sub>S</sub> and predominance of the mono-glycosylated form) and accounts for 10% of all sCJD. The mean age at onset is 65 years with a range of 36-83 years while the disease...
Table 1. Pathological features of sCJD molecular subtypes.

| sCJD molecular subtypes | % of cases | Median age at onset (years) | Duration (months) | Main neuropathological alterations |
|-------------------------|------------|----------------------------|-------------------|-----------------------------------|
| MM1                     | 67         | 66                         | -4                | Diffuse spongiosis with small vacuoles affecting the neocortex, striatum and cerebellar cortex. Synaptic pattern of PrPSc deposition |
| MV1                     | 3          | 66                         | -4                |                                    |
| VV1                     | 1          | 44                         | -21               | Severe spongiosis with fine vacuoles in the cerebral cortex and striatum. Punctate pattern of PrPSc deposition |
| MM2-thalamic            | 2          | 52                         | -16               | Atrophy of the thalamus and inferior olivary nuclei with spongiform alterations confined to the cerebral cortex. Weak and synaptic pattern of PrPSc deposition |
| MM2-cortical            | 2          | 64                         | -16               | Severe spongiosis with large confluent vacuoles predominantly in cerebral cortex and striatum. Perivascular and coarse pattern of PrPSc deposition |
| MV2                     | 10         | 65                         | -17               | Diffuse and confluent spongiosis similar to W2 subtype. Amyloid Kuru plaques in the molecular and granular layer of the cerebellum. |
| VV2                     | 15         | 64                         | -7                | Spongiform changes found in the cerebellum, striatum, thalamus and brainstem. Plaquelike and perineuronal pattern of PrPSc deposition |

MM1, Methionine/Methionine – PrPSc type 1; MV1, Methionine/Valine – PrPSc type 1; VV1, Valine/Valine – PrPSc type 1; MM2-T, Methionine/Methionine – Thalamic PrPSc type 2; MM2-C, Methionine/Valine – Cortical PrPSc type 2; MV2, Methionine/Valine PrPSc type 2; VV2, Valine/Valine PrPSc type 2.

V1 subtype

V1V1 is the rarest subtype of sCJD representing 1% of the total cases. The Western blot analysis shows type 1 PrPSc with a prevalence of the mono-glycosylated isoform. Patients are relatively younger (mean age at onset 44 years) compared to other sCJD subtypes with a mean duration of 21 months (range 17-42 months). Early symptoms include psychiatric or cognitive abnormalities that evolve in extrapyramidal signs and ataxia while myoclonus was observed only in a few patients. Massive spongiform lesions affect the cortico-striatal regions while other subcortical regions and cerebellum are almost spared. Although the severe spongiform changes observed in VV1 patients, PrPSc immunochemistry shows faint punctate staining confined in the cerebral cortex.

Mixed subtypes

Type 1 and type 2 PrPSc have been found to co-exist in about 35% of sCJD cases and may be present in the same or distinct anatomical regions of the same patient. This finding is more frequent in MM (43%) than MV (23%) and VV (15%) cases. The predominance of PrPSc type 1 or 2 influences the clinical and neuropathological phenotype of the disease. The MM1+2 cases mimic the clinical phenotype of MM1 while the PrPSc deposition is a combination of the typical neuropathological features of MM1 and MM2 (synaptic and perivascular patterns, respectively). Conversely, VV1+2 subjects are similar to VV2 sCJD cases in terms of clinical and neuropathological features.
Clinical challenges

The clinical diagnosis of sCJD is particularly challenging especially in the early stages of the disease. It relies on defined criteria that classify the disease as possible or probable. Several clinical, instrumental and laboratory tests are commonly used to formulate an in vivo diagnosis of sCJD: electroencephalogram (EEG),

magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) biomarkers analysis. Several CSF biomarkers have been investigated including the 14-3-3 protein, total tau (t-tau) and phosphorylated tau (p-tau) proteins, neurofilament light chain (NFL), neuron-specific enolase and α-synuclein. The most reliable and commonly used are 14-3-3 and t-tau.

14-3-3 protein is a biomarker of neuronal cell death and therefore it is not specific for prion diseases. It is commonly reported to possess an average sensitivity of 85–95% and a specificity of 40–100%.42–44 It has been used in the 14-3-3 as a biomarker for prion diseases in that the fact that its elevation is common in some neurologic and neurodegenerative diseases including herpes simplex encephalitis, other encephalitis, intracerebral metastases, metabolic encephalopathy, hypoxic brain damage, dementia with Lewy bodies (DLB) and Alzheimer’s disease (AD). Therefore, 14-3-3 analysis may increase the probability of CJD when other clinical features are suggestive of prion disease but it cannot be assumed as a specific biomarker.

Increased levels of t-tau (cut off >1300 pg/mL) may identify sCJD patients with a sensitivity of 67–91% and a specificity of 67–95%.46,47,53–56 This measurement helps to differentiate sCJD from AD. Indeed, t-tau was 3.1 times higher in sCJD compared to AD and 41 times higher than in healthy subjects. Recently, the ratio t-tau/p-tau was found elevated in sCJD patients with a specificity of 94–97% and a sensitivity ranging from 75–94%.

Among other CSF biomarkers proposed for prion disease diagnosis, NFL has been reported to be significantly elevated in sCJD compared to other neurodegenerative disorders like AD, DLB, frontotemporal dementia and vascular dementia. However, despite increased NFL levels enable discrimination of sCJD from normal controls, they do not consent accurate discrimination between sCJD and other rapidly progressive dementias, neurodegenerative dementia and neurological diseases with dementia syndromes. Recently, serum NFL analysis has been suggested as a diagnostic marker for prion diseases showing similar sensitivity and specificity to CSF markers in differentiating sCJD from healthy subjects.

α-synuclein (α-syn) is commonly used as a biomarker for a group of diseases known as α-synucleinopathies, which includes, among the others, Parkinson’s disease (PD) and dementia with Lewy bodies (DLB), but its usefulness for CJD diagnosis has been recently investigated. Two studies reported that total α-syn (t-α-syn) was specifically elevated in CSF of sCJD patients compared to control subjects. Similarly, the phospho-serine-129 α-synuclein (p-α-syn) was found elevated in the CSF of sCJD patients compared to PD, DLB and neurological controls. A combined analysis of both markers, showed 90.5% sensitivity and 97.6% specificity for sCJD diagnosis. Other CSF and serum biomarkers of prion diseases, including the neuron specific enolase (NSE), the S100B protein, SERPINA3 and thymosin β4 are currently under investigation. Unfortunately, although useful for the clinical diagnosis of CJD, CSF biomarkers are not disease-specific.

The definite diagnosis depends on post-mortem examination of the brain aimed at identifying and characterizing the disease-specific biomarker of prion diseases, the PrP. Through a combination of biochemical (e.g., Western blot after PK digestion), immunohistochemical and genetic analyses it is possible to identify the specific sCJD subtype. Thanks to the recent development of the ultrasensitive seeding aggregation assays, named Real-Time Quaking Induced Conversion (RT-QuIC) and Protein Misfolding Cyclic Amplification (PMCA) the diagnostic accuracy of prion diseases has been significantly increased. In particular, the PMCA enabled efficient detection of traces of PrPSc in the CSF, urine and blood of patients with variant CJD (vCJD), which is related to the consumption of foodstuff obtained from cattle affected by bovine spongiform encephalopathy. However, this technique, has never been able to efficiently detect PrPSc associated with sCJD. In contrast, the RT-QuIC has been optimized to efficiently detect low amounts of sCJD prions in the CSF, olfactory mucosa and skin samples in a more rapid and safe manner (with respect to PMCA) while requiring a limited handling of the specimens and reducing the risk of their contamination. For this reason, the RT-QuIC has been adopted by several specialized centers for the analysis of biological samples collected from patients with suspected sCJD, as detailed in the next section.
RT-QuIC assay

RT-QuIC is an ultrasensitive technique developed by Atarashi et al. in 2011 in the field of prion diseases. This assay exploits the intrinsic ability of PrPSc to promote the conformational rearrangement of PrPc that can aggregate into amyloid fibrils.90 The assay mimics in vitro the process of PrPc misfolding and aggregation which occurs in vivo. Recombinant PrPc (recPrP) with the amino acid sequence of different species can be used as a reaction substrate. The addition of traces of PrPc to the reaction substrate induces its aggregation and the kinetics of this process can be monitored in real-time by using a fluorescent dye, named Thioflavin-T (ThT).91 In general, each sample is analyzed in quadruplicates monitored in real-time by using a multi-well plate.92 The samples are subjected to cyclic phases of incubation and shaking using a dedicated fluorescence microplate reader.93 In the presence of PrPSc, the incubation phase stimulates the formation of recPrP amyloid fibrils, while the shaking phase permits the fragmentation of the aggregates into smaller units capable to recruit and convert further recPrP into new amyloid fibrils.90

The in vitro aggregation process can be represented on a cartesian plane where fluorescence is plotted against time generating a kinetic curve characterized by three phases: i) a lag phase, where PrPSc interacts with recPrP and induces this latter to misfold ii) a growth phase, where misfolded recPrP aggregate to form oligomers and small amyloid fibrils sensitive to ThT (exponential increase of fluorescence) and iii) a plateau phase, where almost all recPrP is incorporated into fibrils. Under normal reaction conditions, recPrP spontaneously aggregates while the addition of PrPSc (even in traces) to the substrate significantly accelerates the kinetics of recPrP aggregation (seeding effect) (Figure 3). A sample is considered positive when at least 2 out of 4 replicates show a seeding effect. The RT-QuIC end-products are partially resistant to PK digestion.91

RT-QuIC enabled PrPSc detection in CSF, olfactory mucosa (OM), skin, eye, peripheral nerve, and digestive system of patients with different forms of prion diseases (Table 2).

The assay developed in 2011 was considered the “first generation RT-QuIC” since the analyses were performed at 42°C using the recombinant full-length Syrian Hamster prion protein (recSHa23-231) as reaction substrate. With this experimental setting it was possible to detect PrPSc in the CSF of a series of Japanese subjects with sCJD and 30 Australian sCJD patients with 90% sensitivity and 100% specificity.92

One year later, the analyses of 123 patients with neuropathologically confirmed sCJD showed that RT-QuIC was able to identify PrPSc in CSF with a sensitivity of 91% and specificity of 98%.93

In 2014, Orrù and colleagues94 performed RT-QuIC analysis of OM and CSF collected from living patients with possible or probable clinical diagnosis of CJD. The RT-QuIC analysis of OM identified 30 out of 31 sCJD patients with a sensitivity of 97% and specificity of 100% while the analysis of CSF showed less sensi-

Table 2. Specificity and sensitivity of 1st and 2nd generation of RT-QuIC.

| Samples | Year | Reference | Substrate recPrP | Sensitivity % | Specificity % |
|---------|------|-----------|------------------|---------------|---------------|
| CSF     | 2011 | Atarashi et al.95 | recSHa (23-231)  | 91.5          | 100.0         |
|         | 2012 | McGuire et al.96 | recSHa (23-231)  | 88.0          | 99.0          |
|         | 2014 | Orrù et al.97   | recSHa (23-231)  | 77.0          | 100.0         |
|         | 2015 | Cramm et al.98  | recSHa (23-231)  | Not reported  | Not reported  |
|         | 2015 | Orrù et al.97   | recSHa (23-231)  | 95.8          | 100.0         |
|         | 2016 | Orrù et al.97   | recSHa (23-231)  | 85.0          | 99.0          |
|         | 2016 | Grovenman et al.99 | recSHa (23-231)  | 72.5          | 100.0         |
|         | 2016 | Grovenman et al.99 | recSHa (23-231)  | 93.8          | 100.0         |
|         | 2016 | Park et al.100  | recSHa (23-231)  | 76.5          | 100.0         |
|         | 2016 | McGuire et al.96 | recSHa (23-231)  | 100.0         | 100.0         |
|         | 2017 | Franceschini et al.101 | recSHa (23-231)  | 97.2          | 100.0         |
|         | 2017 | Bongianni et al.102 | recSHa (23-231)  | 71.4          | 100.0         |
|         | 2017 | Bongianni et al.102 | recSHa (23-231)  | 82.6          | 100.0         |
|         | 2017 | Lattanzio et al.103 | recSHa (23-231)  | 82.1          | 99.4          |
|         | 2017 | Fouta et al.104  | recSHa (90-231)  | 92.0          | 98.5          |
|         | 2017 | Fouta et al.104  | recSHa (90-231)  | 95.0          | 100.0         |
|         | 2018 | Rudge et al.105  | recSHa (23-231)  | 89.0          | 100.0         |
|         | 2018 | Hermann et al.106 | recSHa (23-231)  | 97.0          | 100.0         |
|         | 2019 | Abu-Rumieleh et al.107 | recSHa (23-231)  | 82.5          | 100.0         |
|         | 2019 | Abu-Rumieleh et al.107 | recSHa (90-231)  | 97.4          | 100.0         |
|         | 2020 | Fiorini et al.108 | recSHa (90-231)  | 96.0          | 100.0         |
|         | 2020 | Rhoads et al.109 | recSHa (90-231)  | 90.3          | 98.5          |
|         | 2020 | Xiao et al.110   | recSHa (90-231)  | 96.7          | 100.0         |
| OM      | 2014 | Orrù et al.111  | recSHa (23-231)  | 97.0          | 100.0         |
|         | 2017 | Bongianni et al.102 | recSHa (90-231)  | 92.0          | 100.0         |
|         | 2020 | Fiorini et al.108 | recSHa (90-231)  | 91.4          | 100.0         |
| Skin    | 2017 | Orrù et al.111  | recSHa (23-231)  | 100.0         | 100.0         |
|         | 2020 | Mammana et al.112 | recSHa (23-231)  | 89.0          | 100.0         |
| Eye     | 2018 | Orrù et al.111  | recSHa (90-231)  | 100.0         | 100.0         |
| PN      | 2019 | Baiardi et al.111 | recSHa (90-231)  | 100.0%        | 100.0%        |
| DS      | 2019 | Satoh et al.111 | not reported     | 100.0%        | not reported  |

CSF, cerebrospinal fluid; OM, olfactory mucosa; PN, peripheral nerve; DS, digestive system; recSHa23-231, recombinant full-length Syrian hamster prion protein; recSHa90-231, recombinant N-terminally truncated Syrian hamster prion protein.

[European Journal of Histochemistry 2021; 65(s1):3298]
Correlations of RT-QuIC results with neuropathological findings

To date, only few studies have investigated whether there is a correlation between the RT-QuIC results and the phenotypes of sCJD. In the case of CSF samples, the sensitivity of RT-QuIC was found to be high in the most common MM1/MV1 and VV2 sCJD cases, while it was lower in MV2 cases (75–93%). In other rare subtypes, including VV1 and MM2, the sensitivity was found to range between 0-100% and 44-78%, respectively. In these latter cases, the limited amount of CSF, hampered the possibility to properly evaluate the diagnostic accuracy of the assay. In 2016, Foutz et al. observed a correlation between RT-QuIC kinetics and sCJD subtypes. In particular, they observed that MM1 cases had significantly shorter lag phase and higher fluorescence values compared to MM2 cases, and these findings enabled discrimination of both phenotypes with an accuracy of 95%. At the same time, the extended lag phase and lower fluorescence intensities allowed to differentiate VV1 to VV2 individuals with an accuracy of 80%. MV1, MV2, and mixed type sCJD cases did not show significant differences in terms of lag phases or fluorescence intensities.

Recently, Piconi et al. subjected to PK digestion the RT-QuIC products obtained from the analysis of brain homogenates (BH) and CSF of patients with the six phenotypes of sCJD. In this case, regardless of the sCJD subtype, all samples displayed PK-resistant signal characterized by similar electrophoretic mobility and banding profile, even when challenged with several anti-PrP antibodies. Thus, in contrast to the work of Foutz, they could not identify peculiar features useful to distinguish the six sCJD subtypes. For this reason, the possibility to identify sCJD subtypes by RT-QuIC remains to be clearly elucidated. Very recent findings show that formalin fixed brains are capable to exert an efficient seeding activity by RT-QuIC, using both animal and human specimens (personal communication).

Conclusions

Currently, the RT-QuIC test represents the most reliable and powerful tool for the early detection of PrPSc in peripheral tissues of patients with a suspected clinical diagnosis of sCJD. The reason for the rapid growth of RT-QuIC use in the clinical practice, although still confined to specialized laboratories, lays in the fact that it is not invasive for the patients, has a relatively low cost and a high predictive value. Among the advantages, the method is not time-consuming and enables the analysis of a huge number of samples in a relatively short period of time. Overall, these characteristics support the choice by WHO to include the CSF RT-QuIC test in the diagnostic criteria for sCJD. As previously mentioned, only few specialized laboratories have adopted the RT-QuIC technology. However, the assay is relatively easy to learn and can be rapidly used by trained personnel, thus consenting its widening in other centers specialized in the diagnosis of neurodegenerative diseases associated with protein misfolding. Future multi-center trials will consent to verify the robustness of the RT-QuIC for the analysis of peripheral tissues (e.g., OM, skin) and to further explore the potential of this assay to stratify patients in their early disease stage.

References

1. Ladogana A, Puopolo M, Croes EA, Badka H, Jarius C, Collins S, et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. Neurology 2005;64:1586–91.
2. Imam M, Mahmood S. An overview of human prion diseases. Virol J 2011:8:559.
3. Heidenhain A. [Klinische und anatomische Untersuchungen über eine eigenartige organische Erkrankung des Zentralnervensystems im Præsensium]. [Article in German]. Z Gesamte Neurol Psy 1929;118:49–114.
4. Mittal M, Hammond N, Husmann K, Lele A, Pasnoor M. Creutzfeldt-Jakob disease presenting as bulbar palsy. Muscle Nerve 2010;42:833–5.
5. Alema G, Bignami A. [Subacute degenerative presenile polioencephalopathy with akinetic stupor and decorticate rigidity with myoclonus (“myoclonic” variety of the Jakob-Creutzfeld disease)]. [Article in Italian]. Riv Sper Freniatr Med Leg Alien Ment 1959;83:S1485–623.
6. Nowacki P, Kuczyckyi J, Narolewska A, Grzezec H. Amyotrophic form of Creutzfeldt-Jakob disease with rapid course in 82-year-old man. Folia Neuropathol 2000;38:161–3.
7. Stahl N. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 1987;51:229–40.
8. Castle AR, Gill AC. Physiological functions of the cellular prion protein. Front Mol Biosci 2017;4:19.
9. Turk E, Teplow DB, Hood LE, Prusiner SB. Purification and properties of the cellular and scrapie hamster prion proteins.
28. Cassard H, Huor A, Espinoza JC, Douet JY, Lugan S, Aron N, et al. Prions from sporadic Creutzfeldt-Jakob disease patients propagate as strain mixtures. MBio 2020;11:e00393-20.

29. Puoti G, Giaccone G, Rossi G, Canciani B, Bugiani O, Tagliavini F. Sporadic Creutzfeldt-Jakob disease: Co-occurrence of different types of PrPSc in the same brain. Neurology 1999;53:213-6.

30. Zerr I, Schulz-Schaeffer WJ, Giese A, Bodemer M, Schröter A, Henkel K, et al. Current clinical diagnosis in Creutzfeldt-Jakob disease: Identification of uncommon variants. Ann Neurol 2000;48:323-9.

31. Parchi P, de Boni L, Saverioni C, Denen ML, Butts JD, et al. A subtype of sporadic prion disease mimicking fatal familial insomnia. Neurology 1999;52:1757-63.

32. Puoti G, Bizzzi A, Forlomi G, Safar JG, Tagliavini F, Gambetti P. Sporadic human prion diseases: molecular insights and diagnosis. Lancet Neurol 2012;11:618–28.

33. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

34. Rees CR, Inglehearn CF, Forlomi G, Krüger D, Mandell J, et al. Co-occurrence of PrPSc types: an updated classification. Acta Neuropathol 2009;118:659–71.

35. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

36. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

37. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

38. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

39. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

40. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

41. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

42. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

43. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

44. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

45. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.

46. Bate C, Nolan W, McHale-Owen H, Williams A. Sialic acid in Creutzfeldt-Jakob disease. PLoS Pathog 2011;11:74.
Alzheimer’s disease. Neurobiol Aging 2009;30:1834–41.
47. Sanchez-Juan P, Green A, Ladogana A, Cuadra Corrales N, Saanchez-Valle R, Mitrova E, et al. CSF tests in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology 2006;67:637–43.
48. Stoeck K, Sanchez-Juan P, Gawinecka J, Green A, Ladogana A, Pocchiari M, et al. Cerebrospinal fluid biomarker support- ed diagnosis of Creutzfeldt-Jakob disease and rapid dementia: a longitudinal multicentre study over 10 years. Brain 2012;135:3051–61.
49. Sato J, Kurohara K, Yukiike M, Kuroda Y. The 14-3-3 protein detectable in the cerebrospinal fluid of patients with prion-unrelated neurological diseases is expressed constitutively in neurons and glial cells in culture. Eur Neurol 1999;41:216–25.
50. Zerr I, Bodemer M, Gefeller O, Otto M, Poser S, Wiltfang J, et al. Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. Ann Neurol 1998;43:32–40.
51. Chapman T, McKeel DW, Morris JC. Misleading results with the 14-3-3 assay for the diagnosis of Creutzfeldt-Jakob disease. Neurology 2000;55:1396–8.
52. Muayqil T, Gronseth G, Camicioli R. Evidence-based guidelines for prion diseases. Sci Rep 2017;7:15637.
53. Schmitz M, Villar-Piqué A, Karsanidou A, Dafou D, Xanthopoulos K, et al. Cerebrospinal fluid neurofilament light in suspected sporadic Creutzfeldt-Jakob disease. J Clin Neurosci 2019;60:124–7.
54. Chapman T, McKeel DW, Morris JC. Misleading results with the 14-3-3 assay for the diagnosis of Creutzfeldt-Jakob disease. Neurology 2000;55:1396–8.
55. Chapman T, McKeel DW, Morris JC. Misleading results with the 14-3-3 assay for the diagnosis of Creutzfeldt-Jakob disease. Neurology 2000;55:1396–8.
to the clinical differentiation of Creutzfeldt-Jakob disease. Arch Neurol. 2012;69(7):686-72.

78. Barria MA, Libon A, Mitchell G, Head MW. Susceptibility of human prion protein to conversion by chronic wasting disease prions. Emerg Infect Dis 2018;24:1482-9.

79. Moda F, Gambetti P, Notari S, Concha-Marambio L, Catania M, Park K-W, et al. Prions in the urine of patients with variant Creutzfeldt-Jakob disease. N Engl J Med 2014;371:530-9.

80. Concha-Marambio L, Pritzkov S, Moda F, Tagliavini F, Ironside JW, Schulz PE, et al. Detection of prions in blood from patients with variant Creutzfeldt-Jakob disease. Sci Transl Med 2016;8:370ra183.

81. Belondrade M, Nicot S, Mayran C, Bruyere-Ostells L, Almela F, Di Bari MA, et al. Sensitive protein misfolding cyclical amplification of sporadic Creutzfeldt-Jakob disease prions is strongly seed and substrate dependent. Sci Rep 2021;11:4058.

82. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi M, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. Nat Med 2011;17:175-8.

83. Franceschini A, Baiardi S, Hughson AG, McKenzie N, Moda F, Rossi M, et al. High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions. Sci Rep 2017;7:10655.

84. Orrù CD, Bongianni M, Tonoli G, Ferrari S, Hughson AG, Groveman BR, et al. A test for Creutzfeldt-Jakob disease using nasal brushings. N Engl J Med 2014;371:519-29.

85. Mammana A, Baiardi S, Rossi M, Franceschini A, Donadio V, Capellari S, et al. Detection of prions in skin punch biopsies of Creutzfeldt-akob disease patients. Ann Clin Transl Neurol 2020;7:559-64.

86. Cazzaniga FA, De Luca CMG, Bistaffa E, Consonni A, Legname G, Giaccone G, et al. Cell-free amplification of prions: Where do we stand? Prog Mol Biol Transl Sci 2020;175:325-58.

87. Schmitz M, Cramm M, Llorens F, Müller-Cramm D, Collins S, Atarashi R, et al. The real-time quaking-induced conversion assay for detection of human prion disease and study of other protein misfolding diseases. Nat Protoc 2016;11:2233-42.

88. McGuire LI, Peden AH, Orrù CD, Wilham JM, Appleford NE, Mallinson G, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Ann Neurol 2012;72:278-85.

89. Orrù CD, Groveman BR, Raymond LD, Hughson AG, Nonno R, Zou W, et al. Bank vole prion protein as an apparently universal substrate for RT-QuIC-based detection and discrimination of prion strains. Supattapone S, editor. PLoS Pathog 2015;11:e1004983.

90. Bistaffa E, Vuong TT, Cazzaniga FA, Tran L, Salzano G, Legname G, et al. Use of different RT-QuIC substrates for detecting CWD prions in the brain of Norwegian cervids. Sci Rep 2019;9:18595.

91. Orrù CD, Yuan J, Appleby BS, Li B, Li Y, Winner D, et al. Prion seeding activity and infectivity in skin samples from patients with sporadic Creutzfeldt-Jakob disease. Sci Transl Med 2017;9:eaam7785.

92. Orrù CD, Soldau K, Cordano C, Llibre-Guerra J, Green AJ, Sanchez H, et al. Prion seeds distribute throughout the eyes of sporadic Creutzfeldt-Jakob disease patients. MBio 2018;9:e02095-18.

93. Foutz A, Appleby BS, Hamlin C, Liu X, Yang S, Cohen Y, et al. Diagnostic and prognostic value of human prion detection in cerebrospinal fluid. Ann Neurol 2017;81:79-89.

94. Piconi G, Peden AH, Barria MA, Green AJE. Epitope mapping of the protease resistant products of RT-QuIC does not allow the discrimination of sCJD subtypes. PLoS One 2019;14:e0218509.

95. Hermann P, Appleby BS, Brandel J-P, Soyeux P, Collins S, Geschwind MD, et al. Biomarkers and diagnostic guidelines for sporadic Creutzfeldt-Jakob disease. Lancet Neurol 2020;19:235-46.

96. Rudge P, Hyare H, Green A, Collinge J, Mead S. Imaging and CSF analyses effectively distinguish CJD from its mimics. J Neurol Neurosurg Psychiatry 2018;89:461-6.
111. Hayashi Y, Iwasaki Y, Yoshikura N, Asano T, Mimuro M, Kimura A, et al. An autopsy-verified case of steroid-responsive encephalopathy with convulsion and a false-positive result from the real-time quaking-induced conversion assay. Prion 2017;11:284–92.

112. Hoover CE, Davenport KA, Henderson DM, Pulscher LA, Mathiason CK, Zabel MD, et al. Detection and quantification of cwd prions in fixed paraffin embedded tissues by real-time quaking-induced conversion. Sci Rep 2016;6:25098.

113. Green AJE. RT-QuIC: a new test for sporadic CJD. Pract Neurol 2019;19:49–55.

114. Cramm M, Schmitz M, Karch A, Zafar S, Varges D, Mitrova E, et al. Characteristic CSF prion seeding efficiency in humans with prion diseases. Mol Neurobiol 2015;51:396-405.

115. Orrú CD, Groveman BR, Hughson AG, Zanusso G, Coulthard MB, Caughey B. Rapid and sensitive RT-QuIC detection of human Creutzfeldt-Jakob disease using cerebrospinal fluid. MBio 2015;6:e02451-14.

116. Baiardi S, Redaelli V, Ripellino P, Rossi M, Franceschini A, Moggio M, et al. Prion-related peripheral neuropathy in sporadic Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry 2019;90:424–7.

117. Satoh K, Fuse T, Nonaka T, Dong T, Takao M, Nakagaki T, et al. Postmortem quantitative analysis of prion seeding activity in the digestive system. Molecules 2019;24:4601.