Prodromal biomarkers in fatal familial insomnia: a longitudinal study in humans and mice (ProFFIle)

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The consortium

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External collaborators

Ignazio Roiter Associazione Familiari Insonnia Familiare Fatale, Patient association *Italy*	Gustavo Mostoslavsky Center for Regenerative Medicine (CReM) Boston University School of Medicine, Academia *USA*	David A. Harris Department of Biochemistry, Boston University School of Medicine, Academia *USA*

Patient associations

- Associazione Familiari Insonnia Familiare Fatale (AFIFF)
- Associazione Italiana Encefalopatie da Prioni (A.I.En.P.)
- Fundación Española de Enfermedades Priónicas
- CJK-Initiative
- A.I.G.S.S. association

AFIFF

Associazione Familiari Insonnia Fatale Familiare

Fundación Española Enfermedades Priónicas

Overall aim of ProFFIle

To discover and test potential **predictors of disease onset and progression** in **non-invasive** biological samples of individuals at risk of the genetic prion disease **fatal familial insomnia (FFI)**

Challenges and needs for FFI therapy

1. FFI is caused by the *PRNP* D178N mutation. Dominantly inherited disease with a long silent phase followed by a rapid neurological decline, which invariably leads to death within one year from onset

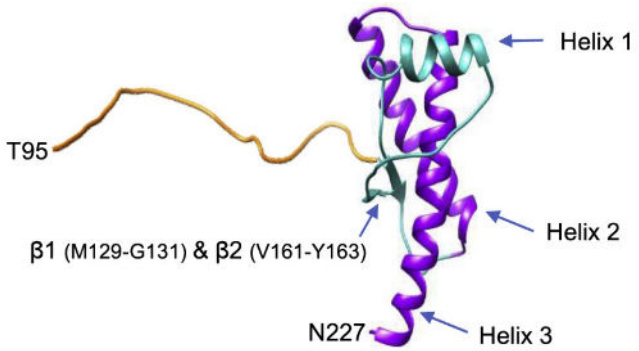
2. Subjects at risk of FFI can be identified by molecular genetic testing decades in advance of symptom onset. However, the lack of prodromal markers leads to clinical diagnosis when substantial neurological damage has already occurred

3. FFI is rare and has highly variable age of disease onset, making it difficult to design preventive clinical trials statistically powered for an endpoint of clinical onset

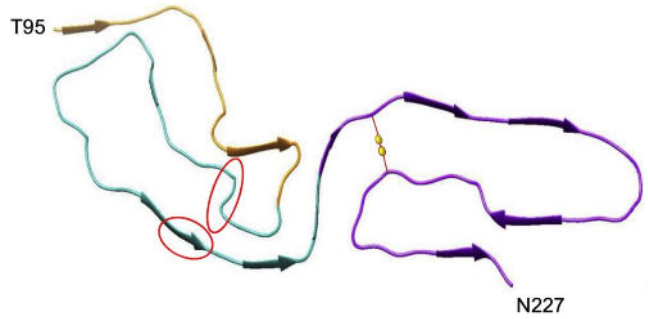
4. For efficacious trial design, it is essential to identify biomarkers that may indicate when to start treatment (proximity biomarkers) and allow monitoring treatment efficacy (surrogate endpoint)

Prion diseases are caused by PrP^{C} to PrP^{Sc} conversion

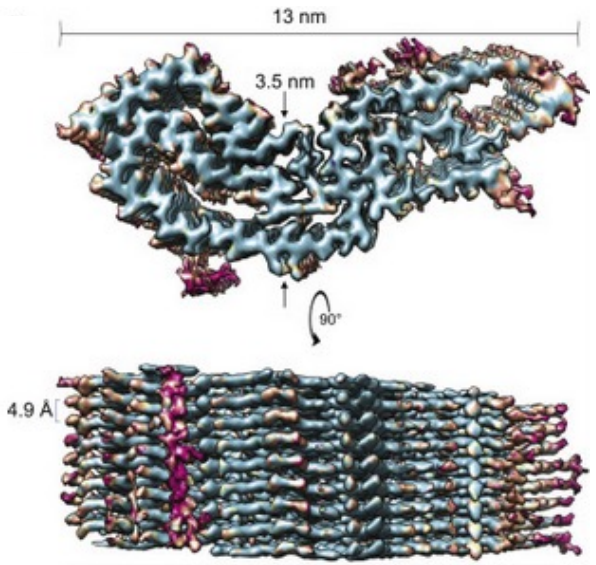
PrP^{C}



PrP^{Sc}
Monomeric unit



PrP^{Sc} fibril

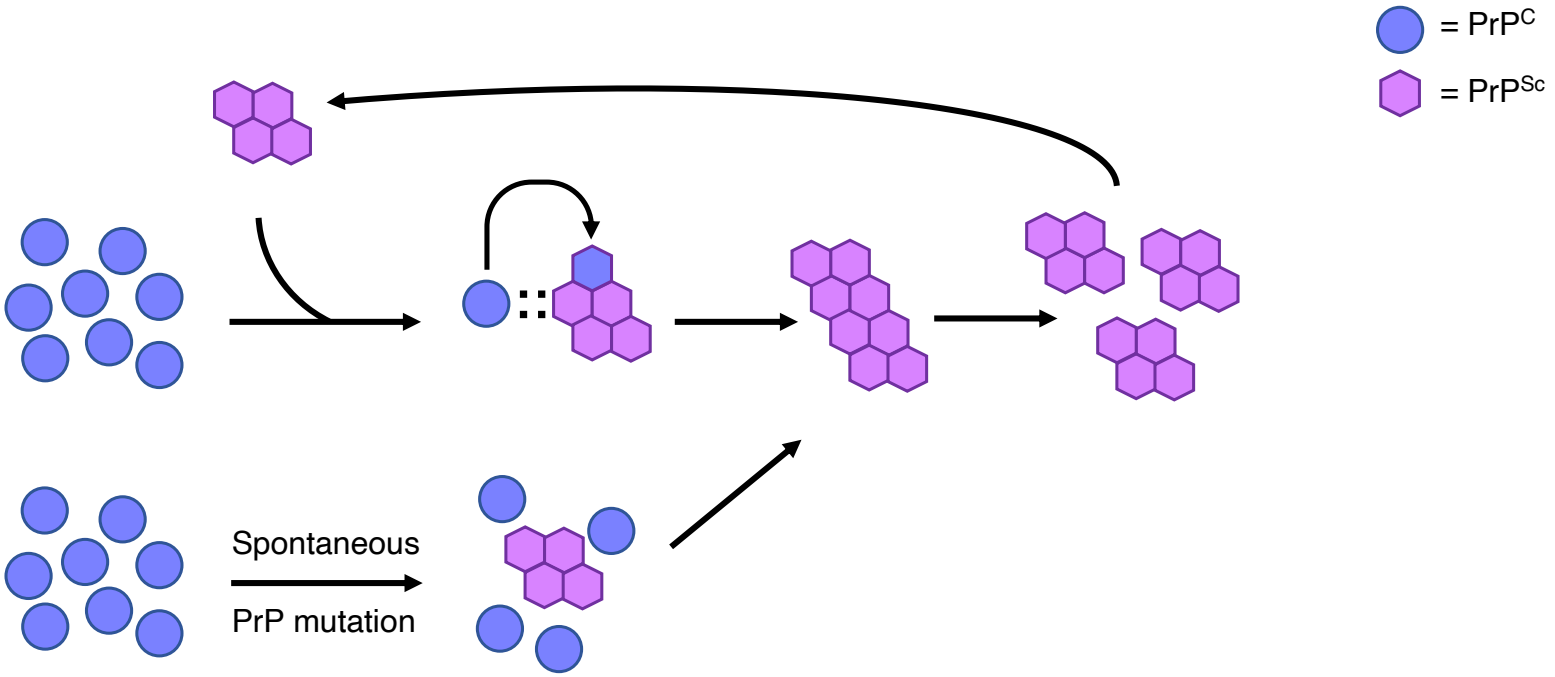


- Cellular protein
- Soluble
- Protease-sensitive

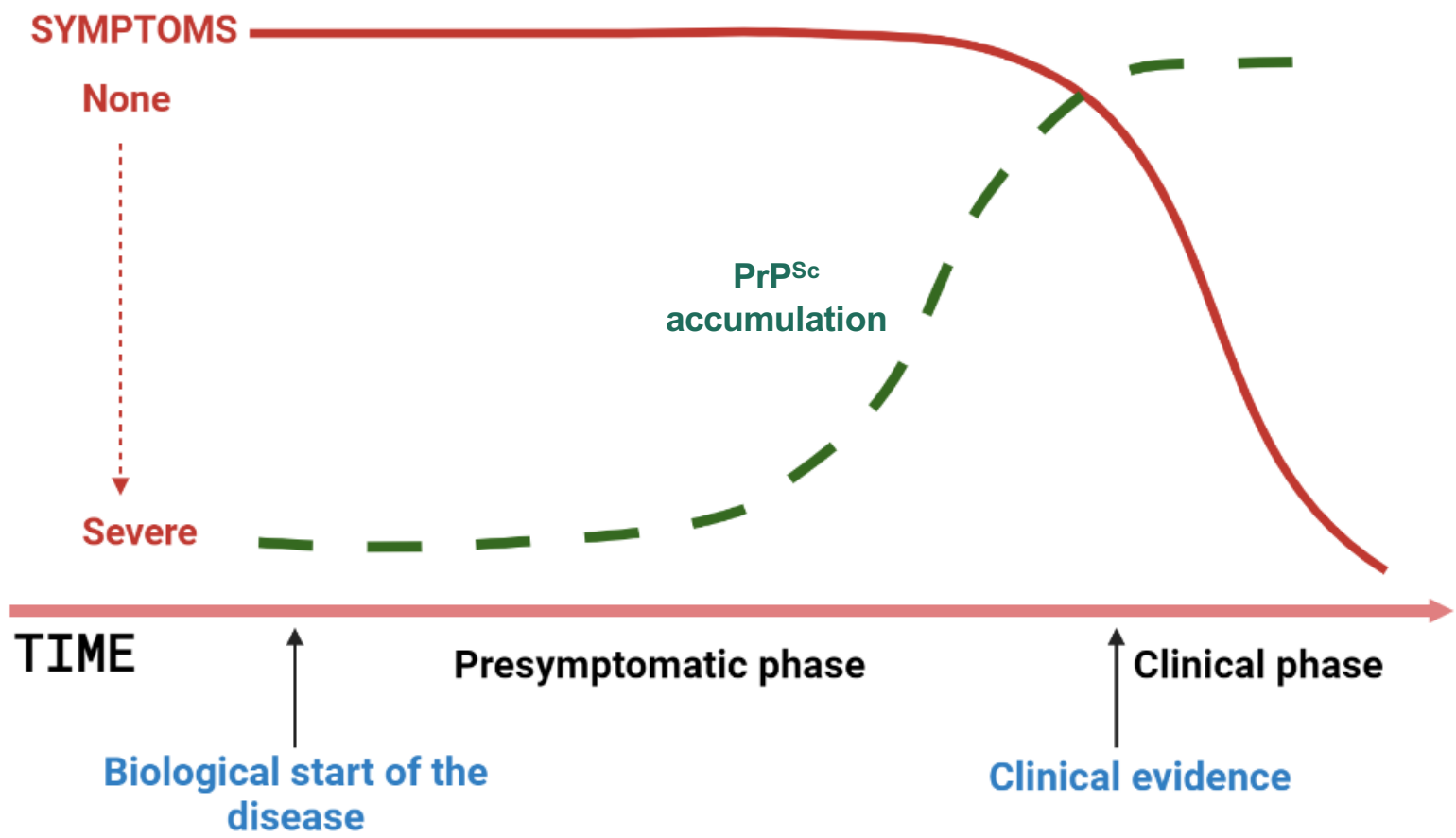
- Disease-specific protein
- Insoluble/aggregated
- Partially protease-resistant
- PIRIBS

Modified from Kraus et al 2021

PrP^{Sc} self-propagates by inducing conversion of PrP^{C}



The pathogenic process precedes the clinical onset



ProFFIle workpackages

- WP1: Measure potential biomarkers (NfL, GFAP, tau) in longitudinal plasma samples of carriers of the FFI mutation
- WP2: Optimize amplification techniques to monitor the emergence of PrP^{Sc} seeding activity in non-invasive FFI samples (blood, urine, olfactory mucosa, tear fluid)
- WP3: Carry out proteomic analysis of brain-derived extracellular vesicles (EVs) purified from the plasma of FFI carriers and non-carrier controls
- WP4: Perform proteomic and metabolomic analysis of EVs purified from the culture medium of neurons derived from iPSCs of carriers of the FFI mutation
- WP5: Characterize a new FFI mouse model and correlate changes in biomarkers levels with the emergence and progression of behavioral disturbances and neuropathological changes
- WP 6: Develop a iPSC-based BBB-on-a-chip model of FFI to investigate FFI-specific pathogenic changes and collect samples for biomarker discovery

ProFFIle results

1. Collaboration with PAs enabled the longitudinal collection of biological samples from well-characterized cohorts of mutation carriers at various disease stages, across different countries

2. Proteomic analysis of plasma samples and brain-derived EVs identified several potential biomarkers which are being validated in longitudinal cohorts

3. Amplification techniques (PMCA and RT-QuIC) have been optimized for detection of FFI prions in blood, urine, olfactory mucosa and tear fluid (see poster #29 by Giuseppe Bufano)

4. A new FFI mouse model was developed by inoculating FFI brain extracts in TgVole mice, allowing longitudinal collection of biological samples

5. A microfluidic BBB-on-a-chip FFI model was developed that indicate alterations in BBB permeability. Outflows were collected for EV isolation for proteomics/metabolomics

Lessons learned and Patient and Public Involvement

1. The international collaboration was vital for the success of the project

2. Involving FFI families across different countries (Italy, Spain, Germany) was crucial for collection of samples

3. The collaboration with Patient Associations (PAs) was also crucial to:
   - address ethical issues
   - raise awareness about the ProFFIle study
   - disseminate the results (social media, news, etc.)

4. ProFFIle promoted the collaboration between PAs of different countries

Partnership between the Italian and Spanish PAs: 2000 Km for research

Thank you for listening!