

ProFFile

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

Poster number 29

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Aims, research questions and working hypothesis

The overall aim of this project is to identify biological markers predictive of onset and progression of fatal familial insomnia (FFI), a rare genetic prion disease caused by the D178N mutation in the *PRNP* gene encoding the prion protein (PrP). Molecular testing can identify individuals at risk of FFI decades in advance of symptom onset. This makes such a subgroup of individuals particularly suited to preventive therapies. However, the highly variable age of disease onset and the lack of defined prodromal biomarkers make it challenging to design efficacious preventive trials.

Brain-derived proteins, such as neurofilament light chain (NfL), tau and misfolded PrP (prion), have been proposed as prodromal biomarkers, but their validity remains to be established. This project aims to: 1) establish a large collection of longitudinal biological samples from carriers of the FFI mutation and non-carrier relatives, through the collaboration with patient associations (PAs); 2) test potential biomarkers in easily accessible biological fluids, such as urine and plasma; 3) carry out proteomic and metabolomic studies on extracellular vesicles (EVs) of brain origin to identify new potential biomarkers; and 4) develop a new animal model of FFI to correlate biomarker levels with the progression of behavioral deficits and neuropathological changes.

Means/methods implemented by the consortium

The consortium engaged PAs to raise awareness about FFI and the goals of the project. Plasma levels of NfL, tau and other potential biomarkers were measured using Simoa, ELISA, AlphaLISA and NuLISA. Protein misfolding cyclic amplification (PMCA) and Real Time Quaking Induced Conversion (RT-QuIC) were used with a large panel of PrP substrates to optimize FFI prion detection in urine and plasma. Targeted and untargeted proteomic and metabolomic approaches were used to analyze the EVs isolated from plasma and the culture media of iPSC-derived neurons. Transgenic mice expressing bank vole PrP were inoculated with FFI brain extracts to generate an animal model of the disease.

Outcomes of the project

Collaboration with PAs enabled the longitudinal collection of biological samples from well-characterized cohorts of mutation carriers at various disease stages, across different countries. Several potential biomarkers were identified and tested in these samples. PMCA and RT-QuIC were optimized for the detection of FFI prions in the urine and plasma by diluting FFI brain extracts into these matrices, achieving detection up to the 10^{-12} dilution. Notably, 2 out of 15 urine samples from carriers at different disease stages tested positive, one from a patient in the clinical phase and one from an asymptomatic carrier. Additionally, a plasma sample from a patient in an advanced disease stage yielded a positive result. The mouse model of FFI was successfully developed and characterized, and longitudinal collection of biological samples initiated.

Significance and impact of the work

The collection of many longitudinal biological samples has enabled numerous studies and analyses. Detecting prions in both urine and plasma samples is a critical step towards achieving the project's ultimate goal of identifying a reliable and specific biomarker of the disease. The development of a FFI mouse model represents a promising platform to investigate pathogenic mechanisms and for preclinical drug testing.

Next steps and future challenges

Further assessment of the specificity and sensitivity of the candidate biomarkers is planned. PMCA and RT-QuIC will be further optimized for testing additional peripheral tissues from mutation carriers at different disease stages. These techniques will be also employed to test the longitudinal urine and blood samples collected from the FFI mouse model.