APPENDIX - 1

STUDY TITLE: Study of potential biological markers and therapeutic targets in post-mortem brain tissues of patients Huntington disease (HD): focus on pediatric HD

START DATE: October 2022

FUNDING SOURCE: Ricerca Finalizzata (till Dec 2023), Ricerca Corrente to CSS (issued by Italian Ministry of Health) (yearly funding), 5x1000 of taxes funding to LIRH Foundation (yearly funding).

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Study Period: October 2022 – present
Background: Pediatric Huntington Disease (PHD) is an aggressive HD phenotype characterized by epileptic features (Cloud et al., Mov Disord 2012; Fusilli et al., Lancet Neurol 2018), early liver steatosis (Squitieri et al., Parkinsonism Relat Disord 2022), early striatum loss of volume (Fusilli et al., Lance Neurol 2018, Tereshchenko et al., Neurology 2019) and severe striatum glucose hypometabolsim (De Volder et al., J Neuro Neurosurg Psych 1990; Zhou et al., Quant Imaging Med Surg 2021). Glucose hypometabolism is a hallmark of several neurodegenerative diseases (Chételat et al., Lancet Neurol 2020) and is caused by altered expression of glucose transporters, specifically GLUT-1 and GLUT-3 (Benarroch, Neurology 2014). In the context of HD, there is only one study by Gamberino et al. that reported a reduced protein expression of GLUT-1 and GLUT-3 in the striatum of advanced stage adult-onset HD patients (AOHD) compared to controls (Gamberino et al., J of Neurochem 1994). So far, there are no studies investigating the involvement of GLUTs in PHD.
Study Population: LIRH Foundation had the opportunity to receive post-mortem brain tissues of frontal cortex and striatum from two PHD, and several early and late onset AOHD and control subjects, from the Department of Pathology, Leiden University Medical Center (LUMC) (The Netherlands). In the past, we already obtained AOHD and control samples by the New York Brain Bank (NYBB), Columbia University, New York (US). LIRH Foundation has been also collecting fibroblast cell lines which were derived from skin biopsies of AOHD, Juvenile-onset HD (JOHD), PHD and control cohorts, in collaboration with CSS and Bambino Gesù Research Hospital.
Objectives: As first stage of our program, we aim to analyze the protein expression of glucose transporters GLUT-1 and GLUT-3 in post-mortem brain and fibroblast cell lines together with the expression of other factors playing a role in the brain glucose activity, such as Hexokinases and Mitochondrial Machinery Complexes (OXPHOS).
Methodology: protein and RNA expression will be studied by Western Blot and qRT-PCR. Immunoistochemistry (IHC) will be also performed to confirm quantitative analyses.
Relevance: Increased disease severity in PHD patients has yet to be fully explained. Investigating, for the first time, the involvement of glucose transporters in PHD, will give new and critical insights in the pathomechanisms related to PHD for therapeutic strategies.