

## **A new universal method for the isolation and deep proteomic characterization of extracellular vesicles to study drug resistance in a paediatric model of epilepsy**

Extracellular vesicles (EVs) are small particles released by cells that carry proteins and genetic material, playing a significant role in cell communication. These vesicles can be found in various body fluids, including blood, making them accessible for study in patients. EVs are involved in numerous physiological and pathological processes, such as immune response, inflammation, and cancer progression. Because of their ability to transfer bioactive molecules between cells, they hold great potential as sources of biomarkers for disease progression or treatment response. Therefore, research on EVs has grown significantly over the past few years.

Epilepsy is a neurological disorder characterized by recurrent, unprovoked seizures. It affects around 1% of the global population, with significant impacts on quality of life. For some patients, managing seizures remains a major challenge. The cost of treating epilepsy is substantial, not only in terms of medical expenses but also considering the broader societal impact. Effective treatments can significantly improve the lives of patients and reduce the overall economic burden. Tuberous sclerosis complex (TSC) is a genetic disorder that in more than 80% of cases leads to epilepsy, affecting children from a young age and very often resistant to first-line antiseizure medication. Therefore TSC is often used as a model to study epilepsy.

Growing literature evidence and findings from our previous work within the EPIMARKER project, which focused on studying drug-resistant TSC patients, suggest that EVs could be pivotal in understanding epilepsy. However, existing methods for isolating EVs are inefficient, costly, and therefore impractical for the large-scale experiments required in clinical studies.

This project aims to address these limitations by developing a novel large-scale method for isolating EVs and establishing a comprehensive mass spectrometry (MS) based protein panel to assess the purity and tissue specificity of EVs. This methodology, together with deep-profiling of EVs proteins, will be used to study plasma EVs from paediatric patients with TSC who are either drug-resistant or responsive to vigabatrin, a medication used to treat seizures. Patients will be examined before the seizures begin, during the first onset of epilepsy, and after a few months of disease treatment.

The anticipated outcomes of this project can significantly advance our understanding of epilepsy progression and the mechanisms behind drug resistance, thereby aiding in the identification of patients at higher risk. Moreover, the methodologies developed through this research, including an efficient protocol for EV isolation and a comprehensive protein panel for EV characterization, can serve as valuable tools for broader biomedical research. This may pave the way for the development of improved diagnostic tools and innovative therapeutic strategies across a wide range of medical conditions.