

I am very grateful to have been awarded with a TRAVEL BURSARY from SSIEM. This allowed a 3-weeks (08/11/2021-26/11/2021) internship in the group of Mitochondrial Pathology and Neuromuscular Disorders from Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain.

My goal was to learn some techniques for functional validation of variants of unknown significance (VUS) detected in the nuclear genome of patients with mitochondrial pathology. During the 3 weeks I practiced the following techniques:

1. **Fibroblast Culture:** Preparation of culture medium; cell count and passage; freezing of cells in liquid nitrogen; freeze cell pellets; preparation of cells for the tests to be carried out.

2. **Mitochondrial Translation:** Analysis of de novo mitochondrial protein synthesis is important to clarify the pathogenicity of novel variants found in patients who have mutations in genes associated with mitochondrial translation. Mitochondrial protein synthesis was analysed in cultured cells upon metabolic labelling with [³⁵S] methionine in the presence of emetine which blocks cytoplasmic translation. The protein concentration was measured by Bradford assay and loaded onto a 15% polyacrylamide gel, containing SDS. The gel was dried and newly synthesized polypeptides were detected by autoradiography.

3. **Study of cell growth in medium with galactose:** In order to assess mitochondrial performance, fibroblasts were exposed to galactose medium, where cells were forced to rely on oxidative phosphorylation for ATP production. The cells grown in galactose rely mostly on oxidative phosphorylation (OXPHOS) instead of glycolysis to produce ATP. However, cells with an impaired OXPHOS system grow poorly in culture medium containing galactose. Cell counts were performed at 48h; 96h; 168h; 216h; 264h.

4. **Western Blot:** To confirm the localization of a protein in the mitochondria we performed subcellular fractionation to isolate the mitochondria from the fibroblasts. Protein lysates from total fraction and mitochondria-enriched fraction were separated on a 10% polyacrylamide gel with SDS followed by Western blotting using primary and secondary antibodies.

The establishment of these functional studies will allow us to clarify the impact of VUS on protein function in order to achieve a definitive molecular diagnosis in our cohort of patients. A molecular diagnosis will allow the clinician to provide a prognosis, an accurate genetic counseling for prenatal diagnosis and lead to personalized treatments.

I am very grateful to the SSIEM for giving me this Travel Bursary for funding this scientific visit. I am also thankful to Mr. Nic Law and Ms. Caroline Hankinson for preparing all necessary business documents.

Finally, I would like to express my gratitude to Dr. Javier Torres for guiding and teaching me all these techniques throughout these intensive 3 weeks. I also thank Dr. Ramon Martí for kindly welcoming me to his research group and all the team members for their hospitality. It was really a pleasure to collaborate and learn with them.

Sincerely,

Lisbeth Silva

Postdoctoral at Research & Development Unit, Human Genetics Department,
National Institute of Health Doutor Ricardo Jorge