# THESIS TOPIC

**FUNDING:**
- □ Requested
- □ Acquired

**Funding origin:**

**Thesis title:** Multiomic approaches of Leber hereditary optic neuropathy physiopathology

**3 keywords:** LHON, metabolomics, fluxomics

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**Unit / team:** INSERM UMR 1083/CNRS 6015 - MITOVASC Physiopathologie MITOchondriale et cardioVASCulaire

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**Socio-economic and scientific context (approximately 10 lines):**

The Leber Hereditary Optic Neuropathy (LHON) is the most frequent inherited mitochondrial disorder, with more than 16,000 patients in Europe, among which 5,000 are blind. Despite the well-known genetic cause related to 3 mitochondrial DNA mutations, leading to the respiratory complex I dysfunction and reduced ATP generation, the metabolic and signaling mechanisms leading to phenotypic expression of the disease are unknown. First, even if all cells types are affected by the mutation, in vivo only the retinal ganglion cell forming the optic nerve is clearly affected.

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**Working hypothesis and aims (approximately 8 lines):**

Cells carrying LHON mutations – available in the team - exhibited general decreased amino acid concentration, and those coming from symptomatic patients have further triggered the endoplasmic reticulum stress response (ERSR). Paradoxically, the protein synthesis inhibitor rapamycin alleviates symptoms associated to complex I dysfunction. The present thesis aims to solve this paradox by examining different aspects of nitrogenous metabolism through metabolomics, (phosphor)-proteomics and fluxomics approaches. Our working hypothesis is that in LHON-carriers, lower amino acids concentration inhibits protein translation, and that in symptomatic patients protein translation is not sufficiently repressed leading to ERSR and eventually to cell death.

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**Main milestones of the thesis (approximately 12 lines):** The thesis project is structured in 3 work-packages (WP)

**WP1- Cell culture, isotopic labelling and sample collection.** Samples from 10 controls and 10 patients carrying a LHON mutation (5 asymptomatic carriers and 5 affected patients) will be generated (including isotopically labeled with D-glucose-1,13C and L-glutamine-15N) with or without the ERSR inhibitor TUDCA (Tauro-ursodeoxycholic acid).

**WP2- Omics and isotope analysis.** Metabolites and proteins will be extracted and analyzed from cell pellets and milieu at different time points after adding the marked and unlabeled substrate. Analytical platforms including nuclear magnetic resonance (NMR), gas and liquid chromatography-mass spectrometry (GC-MS and LC-MS) and isotope-ratio mass spectrometry (IRMS) will enable an optimal coverage of (phosphor) proteome, metabolome and fluxome.

**WP3- Data analysis.** Univariate and multivariate unsupervised and supervised analysis using projection-based methods (PCA and PLS-based methods) will be used for metabolome and (phosphor)-proteome data analysis. Flux calculations based on kinetic measurements will enable measurements of the incorporation rate of amino acids into proteins, from glucose and glutamine to amino acid synthesis and lactate production.

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**Scientific and technical skills required by the candidate (2 lines):** The candidate should have a strong background in cell and molecular biology, with technical expertise in cell culture, biochemical (Western blot, oxygraphy), cell biology (Fluorescence microscopy) and basic statistics.

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3 publications from the team related to the topic (last 5 years):


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**National and international collaborations:**

The project will benefit from collaboration with recognized facilities for (phosphor)-proteomic analysis (platform PAPPSO located in Gif-sur-Yvette, Le Moulon) and NMR analysis (Moltech Anjoz). WP 2 and 3 will be carried on in close collaboration with a foreign partner at Australian National University (Professor Guillaume TCHERKEZ). Professor TCHERKEZ is a recognized expert in isotopes utilization in metabolism. This outstanding linkage between MITOVASC and ANU has been recognised by the scheme FASIC 2018, an international linkage between France and Australia, through Campus France and the French Ministry of Foreign Affairs.