Cell compartment-specific metabolic alterations in a mouse model of Leigh syndrome

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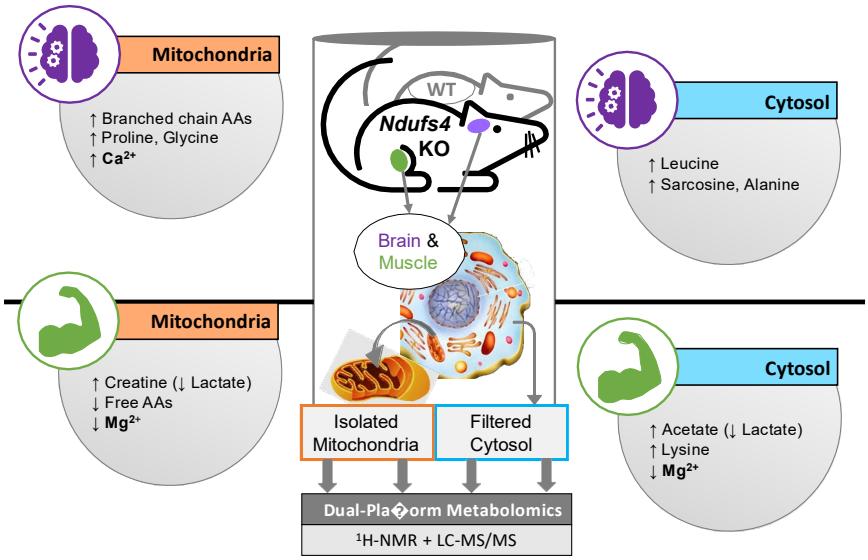

Graphical summary: Ndufs4 knockout (KO) Leigh syndrome (LS) model mice and healthy controls (WT; n = 8 each) were used to explore metabolic compartmentalisation in the tissues most affected by the resulting neuromyopathy. Whole brain and skeletal muscles were harvested post euthanasia, wherefrom mitochondria and cytosol were isolated via immunopurification and reverse centrifugal filtration, respectively. LC-MS/MS and ^{1}H-NMR analyses of the resulting fractions revealed compartment-specific and bicompartmental alterations in distinct metabolite and cationic cofactor concentrations between KO and WT. These results both emphasize the role of metabolic compartmentalization in known LS-related metabolic changes, while also providing novel targets for further refined study of sub-cellular metabolism in mitochondrial disease.

Introduction: Mitochondrial complex I deficiency (CID) due to hereditary mutations that incur loss of the CI protein subunit, Ndufs4, predominantly leads to a progressive encephalomyopathy known as Leigh syndrome(LS)[1]. While metabolic compartmentalisation is a key feature of several other mitochondria-related diseases[2], this study aimed to provide the first evidence of compartment-specific metabolic alterations in isolated mitochondria and cytosol from *in vivo* CID models.

Methods: To that end, whole brain and skeletal muscle from late-stage Ndufs4 knockout (KO) mice and age/sex-matched controls (WT) were subjected to a novel mitochondrial immunopurification and cytosolic filtration workflow[3]. Untargeted ^{1}H-NMR and semi-targeted LC-MS/MS metabolomics was applied to the resulting cell fractions, alongside a first application of compartment-specific magnesium and calcium measurement via EDTA chelation and ^{1}H-NMR[4]. Important variables discerning WT and KO samples were selected per tissue fraction by univariate statistics.

Results: A predominant increase in multiple amino acids was observed in whole-brain samples, with a moreprominent effect at the mitochondrial level. The muscle tissue, in contrast, mostly presented significant decreases in core bioenergetic intermediates with compartment-specific distribution. These results implicate altered redox homeostasis and alternate respiratory chain fuelling, alongside disturbed urea cycling and protein turnover[5,6]. Tissue- and compartment-specific metal cofactor alterations emphasized stress response-related calcium redistribution between neural cell compartments[7], as well as showing global magnesium depletion in the muscle tissue.

Conclusion: This study confirms the ability of compartment-specific metabolomics to capture both known and novel perturbations related to CID, while resolving compartmentalization in LS-related metabolic pathways that tissue/cell level metabolomics cannot discern[8].

Keywords: Sub-cellular metabolomics; Mitochondrial disease; Leigh syndrome; Ndufs4; mouse model; mitochondria; cytosol.

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