Systemic and tissue-specific metabolic alterations in a mouse model of Leigh syndrome

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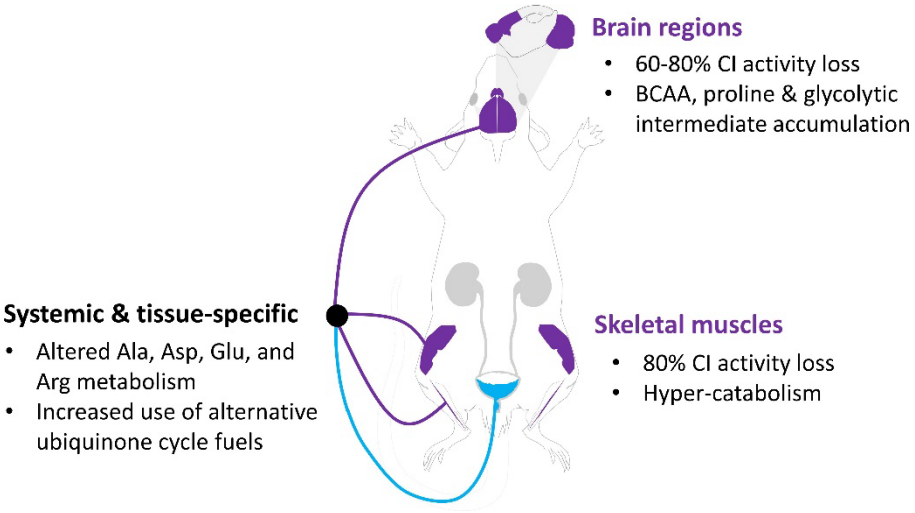

Figure 1: Graphical summary of the systemic and tissue-specific metabolic alterations in a mouse model of LS.

Introduction: The deficiency of complex I (CI) of the oxidative phosphorylation (OXPHOS) system is the most common form of mitochondrial disease (MD) that often presents as Leigh syndrome (LS) — a neurometabolic disorder defined by progressive lesions in specific brain regions. Research on the *Ndufs4* knockout (KO) mouse model of LS has revealed a tissue-specific vulnerability to global CI deficiency with unknown causal mechanisms. The disease most severely manifests in specific brain regions, while others, and peripheral tissues like skeletal muscle, seem relatively unaffected phenotypically. To gain insight into LS,on a tissue-specific and systemic level, we performed metabolomics and OXPHOS enzyme assays on *Ndufs4* KO mice.

Methodology: Semi-targeted LC-MS/MS and untargeted GC-TOF-MS were performed on seven sample types from *Ndufs4* KO (n≥19) vs wild type (WT, n≥20) mice; which include one lesion-resistant and three lesion-prone brain regions; two skeletal muscles of different fibre type composition; and urine. For each sample type, significant changes between genotypes were identified via t-tests and effect size calculations. Sample types were then cross-compared based on effect sizes of unique and shared metabolic alterations. Brain regional and skeletal muscle OXPHOS enzyme activities were measured in post-700×g supernatants from KO (n≥11) vs WT (n≥9) mice and normalised to protein content.

Results: CI activity was significantly decreased (60-80%) in all KO tissues. Among brain regions, a higher residual CI activity and a less perturbed NADH/NAD^+ ratio, correlated with less severe metabolic perturbations in KO mice — with the lesion-resistant region displaying the highest residual CI activity (38% of WT). Brain region-specific alterations included branched-chain amino acid (BCAA), proline, and glycolyticintermediate accumulation. These alterations were more marked in lesion-prone regions, which uniquely displayed lactate accumulation. Elevated levels of classic MD markers were only evident in urinary and brain- regional metabolomes, with skeletal muscles indicating a hyper-catabolic state. *Ndufs4* KO mice further exhibited widespread alterations in alanine, aspartate, glutamate, and arginine metabolism along with decreased levels of alternative ubiquinone cycle fuels.

Discussion and Conclusion: An adaptive rewiring of glutamate and nitrogen metabolism, along with increased oxidation of alternative ubiquinone cycle fuels, seem to be a system-wide response to CI deficiencywhich could serve to compensate for reduced NADH oxidation and electron transport to the ubiquinone pool.While skeletal muscles seem to maintain OXPHOS via hyper-catabolism, brain-regional data suggests pathway congestion — especially in lesion-prone regions with lower levels of residual CI activity. Hence, tissue-specific differences in residual CI activity and the capacity of alternative electron donors or redox- regulatory shuttles, could impact a tissue's ability to bypass CI deficiency. Key pathophysiological targets tofurther investigate in the LS brain include BCAA catabolism, the metabolic sources and fate of α-ketoglutarate in the TCA cycle and the leucine/glutamate/glutamine transport systems between astrocytes and neurons.

Keywords: *Ndufs4* knockout, Leigh syndrome, Complex I deficiency, Metabolomics, Tissue-specificity