Continuous data reveals distinct patterns in metabolite levels along a mitochondrial disease severity scale

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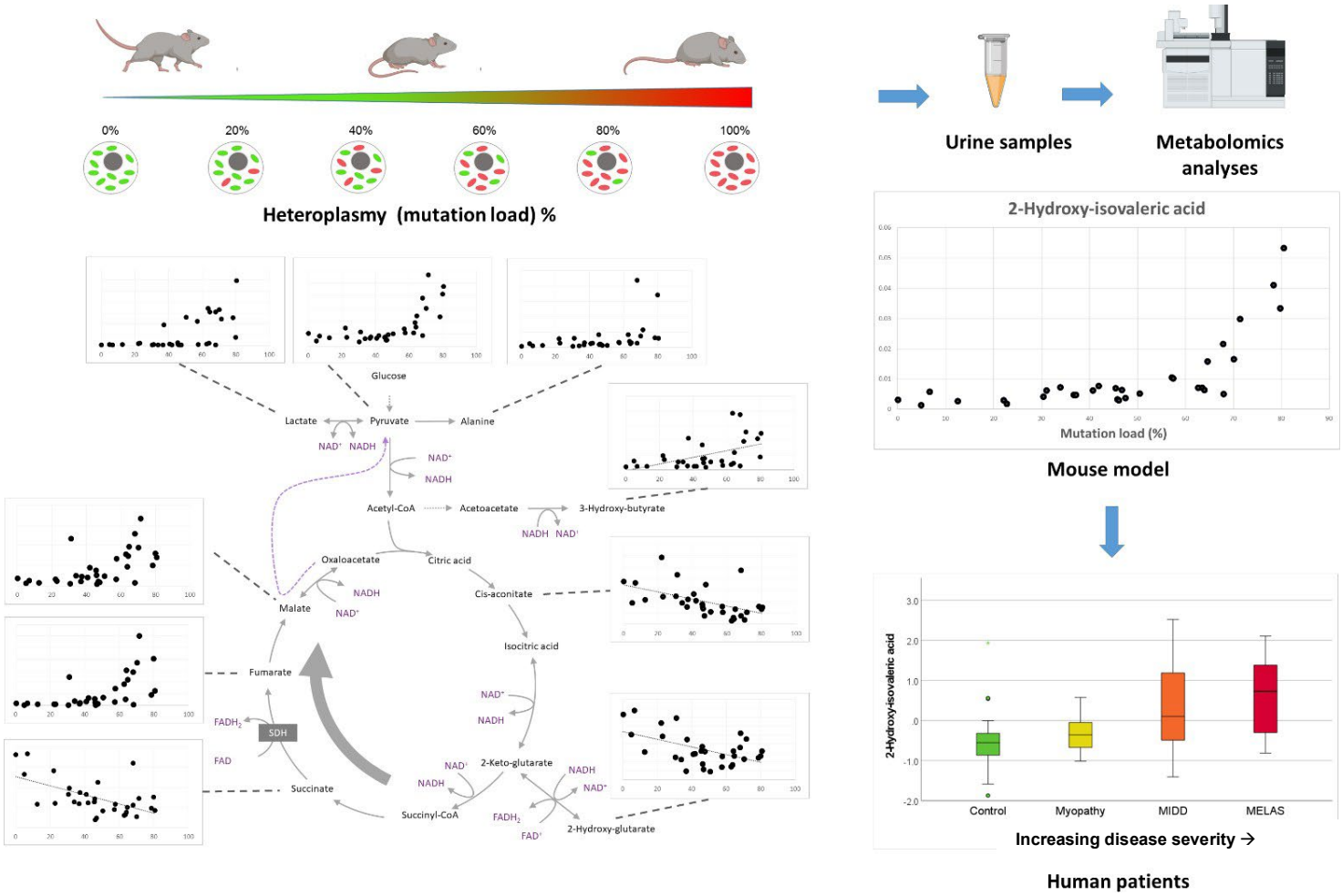

Figure 1: In this study 32 mice with a large mtDNA deletion at heteroplasmy levels ranging from 0 - 80% were used, representing an increase in disease severity. Urine samples were collected and metabolomics analyses performed. The continuous nature of the data allowed us to identify distinct patterns in metabolic perturbations over increasing disease severity, with some metabolites showing a clear threshold effect at around 60% heteroplasmy, while others increased/decreased in a linear fashion. A potential new marker, 2-hydroxy-isovaleric acid, was identified and cross-checked in human mitochondrial disease patients, where it also correlated well with disease severity. MIDD: maternally inherited diabetes and deafness; MELAS: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; SDH: succinate dehydrogenase.

Introduction: Pathogenic mutations / deletions / insertions of mitochondrial DNA (mtDNA) are major causes of primary respiratory chain disorders (RCDs). A common feature of RCDs caused by mtDNA mutations is that a single mutation might manifest in several different distinct clinical phenotypes, while in other cases the same phenotype can be the consequence of different mtDNA mutations, complicating diagnosis and treatment[1].

Methodology: Here we utilised a mouse model that harbours a large 4696 bp deletion in mtDNA, called MitoMiceΔ[2], that present with disease phenotypes characteristic of RCD patients. By analysing urine samples from 32 mice with increasing levels of deletion heteroplasmy (referred to as mutation load), we were able to follow changes in metabolite concentrations across this range.

Results: Several metabolites of the Krebs cycle, lysine, and branched chain amino acids pathways presented with increasing or decreasing concentrations along the disease severity scale caused by increasing mutation load. While some metabolites demonstrated a clear threshold effect at around 60% mutation load, others had a linear pattern. A strong correlation between 2-hydoxy-isovaleric acid and mutation load was seen in the mouse model. This was also reflected in a human cohort of mitochondrial disease patients with an mtDNA mutation that manifests in several clinical phenotypes with different severity scores.

Discussion and Conclusion: Affected metabolites were often associated with NAD^+/NADH dependent dehydrogenases, which suggests a disturbed redox status is driving these observed adaptive changes. The continuous data approach used here is useful to detect early indicators of mitochondrial disease that could be overlooked in classic dichotomous studies. 2-Hydroxy-isovaleric acid could be a possible new marker of severe disease.

References: 1. Grady, J. P. *et al.* 2018. EMBO Mol Med, 10(6). https://doi.org/10.15252/emmm.201708262.2. Inoue, K. *et al.* 2000. Nat Genet, 26(2), 176-181. https://doi.org/10.1038/82826

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