Review of: "Integrated multi-omics reveals anaplerotic rewiring in methylmalonyl-CoA mutase deficiency"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The manuscript by Forny et al presents the integration of multiomics study (genomics, transcriptomics, proteomics and metabolomics) using different models such as primary fibroblast cultures from patients, mouse model and cell models of methylmalonic aciduria to investigate the dysregulation of TCA cycle in methylmalonic aciduria due to MMUT deficiency. Proteomics and transcriptomics analysis performed on selected MMUT fibroblasts (n= 150) and compared to controls (n=80) suggests a disruption of the TCA cycle. This hypothesis was first reinforced by untargeted metabolomics analysis performed in a subset of MMUT fibroblasts and in tissues from a MMUT-mouse model. Flux studies were then performed on a MMUT HEK293 cell model. Incorporation of labelled glutamine and enrichment in TCA cycle intermediates support the hypothesis of an increase of the oxidative utilization the anaplerotic glutaminolysis pathway. Finally, the author produce argument for direct interaction between MMUT and DLST protein. The loss of this interaction may contribute to the TCA cycle disruption.

Methods used are sounds, the number of MMUT fibroblast samples studied (n =150) is large and unique, the different models used are complementary. Results are supported and open new perspectives in understanding the pathophysiological mechanisms and possible future therapeutic approaches in this disease. These data deserve to be published for the medical community.

Major

However, the very first part of the work before the “Multi-layered biology reveals disruption of TCA cycle and associated pathways” chapter is ambiguous.

The authors do not define the selection criteria for the fibroblasts samples included in this first part of the study. It is assumed that these fibroblasts were sent to the laboratory to characterize the aetiology of suspected methylmalonic aciduria based on abnormal urinary excretion of MMA, but this is not specified. Was there an MMA concentration threshold criterion for this inclusion? It seems not because we end up with a heterogeneous group corresponding to different enzyme defects (with indeed a large majority of MMUT forms 150/230) including deficiencies which are today considered as non-diseases (ACSF3) and others with different pathophysiological basis. As a result, the part of the search for association between biochemical phenotype and clinical severity is biased because all fibroblasts samples were included.

It would have been more relevant to present the biochemical phenotyping (propionate incorporation test and MCM activity) and then the genotyping by WGS (both probably performed before this study) to characterised and define the criteria of selection of the fibroblasts samples and then to restrict the analysis to the MMUT forms as it is done since the

third chapter.

Minor

- P6 line 116: Primary fibroblasts culture have a limited life span and their characteristic can be modified with passages. Do the authors take into account this parameter in their study? It should be included in the methods.
- Page 7, line 136 Figure 1h: the data concerning ACSF3 is not related to the rest of the study and should be supplemental data or suppressed
- Extended data Figure 2b: The authors do not clearly define the mut0 and mut- phenotype. How did the author define as a cobalamin-responsiveness in the PI test (threshold)?
- Page 9, line 180-3 and extended data Figure 2f and 2g: why did you include the controls in this correlation to assess the relation between PI and disease severity. This this strongly pulls the correlations and distorts the result, as the controls are normal especially in figure 2f.
- Page 9, line 182-3 and Extended data Figure 4d: it is very difficult to make correlation with the kidney dysfunction as it depend of the age at clinical evaluation, All the 150 MMUT patient have not been evaluated at the same age there is a bias. In addition, the undefined inclusion criteria give the impression that almost half of the MMA do not have a diet and protein restriction!