Point-by-point reply

Reviewer 3

Thank authors for the response and revisions. The addition of Fig 2 and Fig 3 has significantly improved the manuscript, making the results more visual. However, there are still areas for improvement:

1. The first concern I mentioned in the first review report is the lack of innovation in the manuscript, which cannot be resolved simply by referencing the articles I mentioned. I suggest that the authors strengthen the discussion of the novelty of your work in the Introduction and Discussion sections. Based on the existing various researches on the relationship between mtDNA and inflammatory factors in T2D, why is your article valuable?

Thank you for your pertinent suggestion.

The following information has been added:

Introduction:

Despite a large body of evidence with regard to the complex relation between mtDNA abnormalities and atherosclerosis, the literature lacks data concerning the intervention of mtDNA in cerebral vessels structural modifications in type 2 DM. To date, no clinical or experimental study has approached cerebrovascular remodeling in relation to mtDNA variations in the absence of history or symptoms of cerebrovascular disease.

To the best of our knowledge this is the first study which aims to evaluate, from a molecular perspective, a potential association of mtDNA changes in blood and urine and of biomarkers of inflammation with cerebral vessels structural and functional modifications in neurologically asymptomatic patients with type 2 DM.

Discussion:

In our study, serum mtDNA correlated indirectly with IMT-CCAs, PI-ICAs, PI-MCAs, RI-ICAs and with RI-MCAs, thus showing that low levels of serum mtDNA have a negative impact on cerebral vessels by possibly mediating subclinical atherosclerosis and arteriosclerosis, even in normoalbuminuric type 2 DM patients, with no history or symptoms of cerebrovascular disease.

These observations may allow for the assumption that the CCAs, ICAs and the MCAs, and the cerebral vascular bed covered by these vessels and their deriving down-stream branches are very sensitive to mtDNA variations in both serum and urine in the course of type 2 DM. Moreover, these results point to a potential molecular mechanism in which

mtDNA could be instrumental in subtle changes in the cerebral vessels wall from the early stages of DKD.

2. The authors should not hide data that "did not result as significant" from the table. All data, whether significant or not, should be displayed if they are reliable. The lack of "significance" of the data in this study does not mean that it lacks scientific significance.

Thank you for your valuable comment.

A parsimonious model (few variables) is usually preferred over a complex model (many variables). One way to obtain a parsimonious model from a model with many independent variables consists of iteratively removal of the independent variable least significantly related to the dependent variable (i.e., the one with the highest p-value in an analysis of variance table) until all of them are significantly associated to the response variable.

3. There are unnecessary cursor icons entering the figure in the flowchart of Fig 1.

Thank you for your observation.

The cursor icons have been removed from the flowchart.