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### Reply:

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## REPLY:

We have received a letter to the editor regarding our article titled, "MELAS: Phenotype Classification into Classic-versus-Atypical Presentations,"<sup>1</sup> from Josef Finsterer and Sounira Mehri.

Before addressing both the generic and specific comments, we would like to reiterate that our research adhered to rigorous inclusion and exclusion criteria, which encompassed detailed clinical, laboratory, demographic, genetic, and imaging data. To ensure a comprehensive approach, we had mitochondrial experts from pediatric neuroradiology, pediatric neurology, and genetics departments in a high-volume mitochondrial medicine research and clinical referral center at the Children's Hospital of Philadelphia. Additionally, our study incorporated extensive longitudinal data on disease progression, which provided valuable insights into the expected trajectory, the dynamic nature of manifestations, and the severity of the disease. We encourage readers to access the full analyzed data provided in our Online Supplemental Data.

We will first address the generic comments made and then the more specific comments about our article. Answers to some of the questions have been explicitly given in the article, and a full reading of our article and the Online Supplemental Data would have answered them.

First, yes, this study is retrospective as we have explicitly indicated, and that type of study obviously comes with its limitations. As for questions about inclusion and exclusion criteria and the question about how many patients were excluded, they have been delineated in the Materials and Methods section and Fig 2. The fact that this was a single-center study is also explicit, though this is considered a relatively large study with a full set of a variety of variables for a rare disease. As for the comment about being uncontrolled, we are not sure what control group or study design they have in mind, but our study design did not require it. The whole point for a design such as ours and, actually, the main goal and utility of an unsupervised learning statistical analysis such as cluster analysis is in exploratory instances in which a control group is not designated. Thus, a traditional control group would not be required. Perhaps they are envisioning some other type of supervised learning classification study, but that is not ours.

As for the second point, yes, we have not included every factor that could have been potentially conceived, but our multidisciplinary experts did consider a large number of clinical, genetic, and imaging variables that can be accessed in Fig 1, and the choices for the cluster analysis were deliberate to ensure a full set of the most important data for cluster analysis. Other factors were either not considered germane to the aims of our study at this stage or would have precluded full sets of data on these secondary factors that require a much deeper level of genomic analysis.

We had decided not to go on an unfruitful deep fishing expedition to see the influence of details such as haplogroups, other polymorphisms, or other nuclear genes for this study. Some of their other suggestions are not clinically feasible. For example,

mitochondrial DNA (mtDNA) copy number analysis in the United States is only clinically performed in tissue (specifically muscle and liver biopsies). Less than 25% (8/35) of our cohort had muscle biopsy performed, and none had liver biopsy performed, because the diagnostic variant was identifiable in a non-invasive manner at clinically relevant heteroplasmy in most of the cohort (Online Supplemental Data). Given the confirmed genetic diagnosis, an invasive procedure (biopsy) in these individuals was not clinically indicated at the time of their evaluation.<sup>2</sup>

We obviously agree with the generic statistical tenet comment that adding (or subtracting) variables from a cluster analysis can change the results. That possibility is why we were deliberate in our choice of features and variables and did not want to include variables that would have statistically further limited the number of cases considered in the statistical computation of the clusters. One irony in this letter to the editor is that on the one hand there is a criticism that only a small number of patients were included, yet the authors advocate a larger number of variables. That would perhaps deteriorate the robustness of the cluster analysis. We still stand by our contention that this study is a relatively larger study of a rare disease incorporating a wide range of clinical, genetic, and imaging data. Including longitudinal clinical data is another strength of the article. We would certainly agree that a future study based on the new insights provided in our article that can incorporate a deeper granular level of genetic details as noted in the letter could be conceived; and yes, as is generically true for almost all studies, having more patients would help and that could be achieved with multi-institutional studies, particularly for rare disorders.

As for the third point raised that the patients were not diagnosed according to the Hirano or Japanese criteria, we have considered and cited the previously established criteria by Hirano et al and Yatsuga et al.<sup>3,4</sup>

In the fourth point raised, Finsterer and Mehri state that seizures as a manifestation of strokelike episodes were not included in the cluster analysis. They must not have looked at the cluster analysis results. Seizure in strokelike episodes was clearly included (see Fig 3 and the Online Supplemental Data). Their second question about the number of subjects with seizures can be similarly seen in the results.

As for the fifth point about medications in the assessment, their inclusion was not germane to our study, which focused on the evaluation of clustering of clinical and imaging features. Drug safety and toxicity mattered in our study design if they manifested in cardinal clinical or neuroimaging findings. A widely used guide in the mitochondrial disease community for the use of toxic drugs is the Delphi method article by De Vries et al, 2020.<sup>5</sup> The list of drugs stated to be toxic to mitochondria in the letter to the editor is now thought to be outdated, irrelevant to our study design, and referenced by Finsterer.

Finally, we respectfully disagree with their final statement as it relates to our article: "More important than identifying and delineating a syndrome is clarifying the genetic background and pathophysiology of a hereditary disease. Diagnostic and therapeutic management may depend more on etiology and pathogenesis than on phenotypic variability." It is the clinical and imaging features that guide patient evaluations and work-up,

eventually leading to genetic and acquired disease diagnoses, treatment, and monitoring. From our perspective, the delineation of the neuroimaging phenotype is not only a crucial step toward early diagnosis and part of the diagnosis criteria but also represents paramount information that is a cornerstone on which to expand our understanding of the complex etiology and pathogenesis of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). This recognition will contribute to the development of hypotheses regarding pathogenesis, clinical management, and future trials. Additionally, it will enhance our understanding of the clinical presentation and prognosis of MELAS, ultimately aiding in the identification of specific therapeutic interventions. The day-to-day care of patients is often dictated by their clinical status, lab findings, and imaging as well. At least currently, patients do not initially present to their physician with a whole genome or mtDNA sequencing already completed. Generally, one should delineate the phenotype first and then analyze the genome second.

## REFERENCES

1. Alves C, Zandifar A, Peterson JT, et al. **MELAS: phenotype classification into classic-versus-atypical presentations.** *AJNR Am J Neuroradiol* 2023;44:602–10 [CrossRef Medline](#)
2. Mavraki E, Labrum R, Sergeant K, et al. **Genetic testing for mitochondrial disease: the United Kingdom best practice guidelines.** *Eur J Hum Genet* 2023;31:148–63 [CrossRef Medline](#)
3. Hirano M, Ricci E, Koenigsberger MR, et al. **Melas: an original case and clinical criteria for diagnosis.** *Neuromuscul Disord* 1992;2:125–35 [CrossRef](#)
4. Yatsuga S, Povalko N, Nishioka J, et al; Taro Matsuoka for MELAS Study Group in Japan. **MELAS: a nationwide prospective cohort study of 96 patients in Japan.** *Biochim Biophys Acta* 2012;1820:619–24 [CrossRef Medline](#)
5. De Vries MC, Brown DA, Allen ME, et al. **Safety of drug use in patients with a primary mitochondrial disease: an international Delphi-based consensus.** *J Inherit Metab Dis* 2020;43:800–18 [CrossRef Medline](#)

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