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REPLY TO LUTZ-BONENGEL ET AL.:

Biparental mtDNA transmission is unlikely to be the result of nuclear mitochondrial DNA segments

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In Luo et al. (1), we report the transmission of paternal mtDNA in 17 individuals across three unrelated families. In their letter responding to this paper, Lutz-Bonengel et al. (2) argue that these results do not provide sufficient evidence for paternal inheritance of mtDNA. Instead, they propose that these biparental inheritance events are the result of "nuclear elements of mtDNA" [(numts), or nuclear mtDNA segments (NUMTs)]. Although rare, nearly full-length mtDNA insertions have been occasionally observed (3, 4), and there is a possibility that such insertions could superficially resemble a genuine mtDNA.

While we appreciate these concerns, we must point out that our paper merely documents the unusual transmission of apparently full-length paternal mtDNA sequences to offspring. At no point do we propose where these mtDNA sequences are from. However, we do believe that the NUMT hypothesis is extremely unlikely, based on the following observations.

Per our standard protocols (5, 6), we PCR amplified the entire 16.5-kb mtDNA using a pair of back-to-back, nonoverlapping primers, and then sequenced the products by next-generation sequencing. Since these primers face in opposite directions, it is nearly impossible to amplify a linear DNA fragment of full length. In order for a NUMT sequence to amplify, it would have to consist of at least two full-length (or nearly full-length) mtDNA insertions located in close proximity in the same orientation and with the breakpoints situated so that the primers are correctly oriented.

Furthermore, the primer sets in our Method 1 and Method 2 sit in completely different regions of mtDNA. However, the results were always consistent. Thus, the structure of the insertion would have to allow both pairs of primers to be properly oriented for amplification, adding further complexity to the structure of the proposed NUMTs. Finding three such insertion events independently across all three families would be extraordinary. We believe this makes it unlikely that NUMTs are the origin of this phenomenon.

It should also be noted that any single NUMT insertion would be at a massive copy number disadvantage relative to the mtDNA, making it unlikely to be detected using these methods. Our own studies have shown that, at a minimum, there are at least 100 copies of the mitochondrial genome per copy of the nuclear genome (7).

Finally, across these three families, there are four cases in which a heteroplasmic female who inherited mtDNA from her father went on to have children. Eight children were born to these mothers, of which all children we sequenced received the same heteroplasmic mixture of haplogroups observed in their mothers. If these apparent heteroplasmies were caused by mtDNA insertions in the nuclear genome, then one must assume that these insertions exist in a heterozygous state in these four mothers. As a result, the probability of all these children receiving the NUMT insertion would be relatively low. This is another indication that the DNA in question cannot be nuclear in origin.

In the future, we anticipate conclusively settling this question by analyzing additional tissue-specific and single-cell sequencing data from these and other families showing signs of paternal mtDNA transmission.

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The authors declare no conflict of interest.

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