Peer-Review Record

mt-tRNAs in the polymerase gamma mutant heart

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Reviewer 1: Anonymous

Reviewer 2: Anonymous

Round 1

Reviewer 1 Report

The authors show expression of amyloid-associated mt-RNAs is decreased in PLOG mice, suggesting that it could be an adaptive response to mitochondrial dysfunction.

General: The authors' findings are interesting and suggest a novel mechanism of cardiac adaptation against myocardial stress. However, the study appears premature at the present form.

It would be nice if the authors could clarify if decreases in MT-TY are reflected in amyloidogenic potential at the level of proteins.

The fact that mt-tRNAs appear to form functional clusters is interesting. However, more explanation is helpful on how their expression is regulated. The directionality of expression changes in clusters is not necessarily the same as that of nearby genes encoding non-tRNA.

Specific: Since the authors propose that UPRmt induces the regulation of mt-tRNA, it would be essential to show the presence of UPRmt in the RV samples from POLG mice used for RNA seq.

Authors' Response

We thank reviewers for their time and thoughtful critiques. Below, please find a point-by-point response to specific reviewer comments.

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General: The authors' findings are interesting and suggest a novel mechanism of cardiac adaptation against myocardial stress. However, the study appears premature at the present form.

It would be nice if the authors could clarify if decreases in MT-TY are reflected in amyloidogenic potential at the level of proteins.

Yes, we agree it would be worthwhile to test if reduction in MT-TY alleviated proteotoxic stress in the mitochondria. The most direct way to do this would be to specifically target MT-tRNAs of high amyloidogenic potential in a proteotoxic stress environment. Unfortunately, the ability to genetically target mitochondrially encoded genes has only recently been demonstrated for protein coding genes. These efforts were aided by designing siRNAs to identified AGO2 binding peaks (high failure rate without this step, PMC8027830), however no such data are annotated for mt-tRNAs and it is likely that tRNA secondary structures would prevent this approach. Thus, these studies would require significant trial and error to identify effective siRNA sequences with no precedent to suggest it would be successful. CRISPR-type modification of the mitochondrial genome would also be hampered by CRISPR targeting to the mitochondria and heterogeneity of the mitochondria genetic material after targeting (i.e. thousands of mitochondria chromosomes per cell). Another approach could be codon interference through a codon sponge /dominant negative but would also not be ideal due to mitochondrial targeting.

One potentially viable approach may be to target the mitochondrial tRNA synthetase that charges the tRNA with its amino acid. These are more easily targeted genetically as they are encoded in the nucleus and are also more amenable to standard RNAi approaches. We are considering this approach in a cell culture model (i.e. siRNA targeting mt-TyrRS in conditions of mitochondrial stress), though we view this as outside the scope of the current

manuscript as it would take considerable time and effort to establish the necessary models and assays.

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Thank you for your comment. Upon first viewing our data and digging into the literature, we were surprised to learn how little is known regarding dynamic mitochondrial gene expression. Moreover, available research is heavily weighted toward protein coding genes. The following text has been added to the discussion section.

"Though mitochondrial gene expression was first observed in the 1960s, detailed understanding of dynamic mitochondrial gene regulation, particularly for mt-tRNAs is lacking. The basic processes of RNAse-mediated excision from multi-gene/polycistronic transcripts and critical post-transcriptional chemical modifications and amino acid additions are well known (recently reviewed in [36]). However, how specific mt-RNAs of different abundance are generated at steady state or in response to stress has not been robustly characterized. It is likely these differences arise through a number of combined mechanisms that could include regulation of transcriptional efficiency (i.e. more expression of genes found early in the transcript), site-specific regulation of RNAse activity for gene excision, regulation of specific mt-tRNA CCA sequence addition, regulation RNA post-transcriptional modifications, and RNA degradation. All of these factors can be impacted by RNA-binding proteins."

Specific: Since the authors propose that UPRmt induces the regulation of mt-tRNA, it would be essential to show the presence of UPRmt in the RV samples from POLG mice used for RNA seq.

Cannonical UPRmt is not activated in the POLG mutant. Rather, we see gene and protein expression patterns that are consistent with the functional result of UPRmt. Specifically, in the POLG mutant, we reported down regulation of ETC components at the RNA and protein level which also occurs in UPRmt (combining our RNA-seq with publicly available protein data), and a post-transcriptional activation of other chaperones and mitochondrial translation machinery (PMID: 35283176). In UPRmt, this activation of chaperones and mitochondrial translation is mainly by transcription, though in POLG mutants it occurs post-transcriptionally and through different specific proteins of the same pathways/processes. We apologize for the confusion and have reworded text in the abstract and introduction sections to reflect a UPRmt-like response. The following text was added to the introduction section.

"Thus, the UPRmt-like response in POLG does not completely recapitulate UPRmt but is a pattern consistent with reducing proteotoxic stress similar to UPRmt. For instance, POLG mutant show increased protein expression of mitochondrial proteases (AFG3L2, PMPCB), mitochondrial ribosomal proteins (MRPS30, MRPL21, MRPL37, MRPS14, DAP3), translation regulators (ATAD3A, EEFA1A, GUF1), mitochondrial chaperones (PHB1, PHB2, PET100, STOML2), enzymes required for protein folding (PPIB, TMX1), mitochondrial protein importers (TOMM40, MTX1), and factors necessary for complex assembly (ECSIT, DNAJC11, COQ4, COA3, COA5)."

Reviewer 2 Report

The present manuscript investigated the effect of POLG mutation on the expression of mitochondrial-encoded genes in female mice. It was found that the reduced expression of amyloid-associated mt-tRNAs was another indication of adaptive response to mitochondrial dysfunction and it existed a chromosomal location-dependent mt-tRNA regulation. Overall, the authors provided an interesting story, which revealed the mt-tRNAs partner amino acid's amyloidogenic alteration was related to mitochondrial dysfunction.

The only question I am concerned about is what's the conclusion of human subjects of those found in mice.

Authors' Response

Yes, we share this concern which is a regular caveat to pre-clinical research. We have been unable to locate human POLG mutant samples and are not aware of a POLG patient tissue/cell bank, though are in communication with the POLG Foundation to potentially address this issue in future efforts. The following text has been added to the manuscript to at least give consideration to translational aspects.

“While testing to determine if this phenomenon occurs in human samples remains a future aim, remarkable conservation of mitochondrial chromosome gene distribution architecture between mice, humans, and even zebrafish suggests that location dependent regulation of mitochondrial genes would also be conserved. Interestingly, single celled eukaryotes, such as yeast, show far less mt-tRNA gene distribution across the mitochondrial chromosome, potentially implicating candidate regulatory mechanisms in the evolution of multicellular organisms or tissue/organ specialization.”