

POLG Astrocyte Mitophagy Assay State and Reactive-Glia Boundaries

Andrew Friedl

Precision Mito, Florida, USA

Correspondence: afriedl@precisionmito.com

Abstract

Background: POLG encodes the catalytic subunit of DNA polymerase gamma, and POLG disease models now include human iPSC-derived astrocytes, cortical organoids and astrocyte-neuron co-cultures. The unresolved question is whether mitophagy/autophagy readouts in these public papers define a POLG-specific assay-state bottleneck or merely restate generic mitochondrial stress.

Objective: The selected question was whether public POLG astrocyte full texts separate stress-induced mitophagy, basal autophagy-lysosome flux and reactive-astrocyte toxicity, or whether those readouts collapse into one nonspecific mitochondrial dysfunction signal.

Methods: Complete public primary texts and indexed primary records were inspected for three focal POLG astrocyte-lineage papers, with current adjacent primary literature checked for POLG neural iPSC context, astrocyte mtDNA-depletion biology and A1-like reactive astrocyte terminology. A source-comparison pilot coded 17 assay rows from 3 POLG source records across endpoint class, genotype-line context, model, perturbation state and translation limits. No patient-level data, raw-image reanalysis, pooled effect size, treatment ranking or clinical recommendation was generated.

Results: The public matrix retained three non-equivalent endpoint classes: FCCP/PINK1-Parkin/LC3-p62 induced mitophagy and protein-state measurements in the 2021 POLG astrocyte study; mitochondrial dysfunction, A1-like reactivity and neuron co-culture toxicity in the 2024 POLG astrocyte study; and reduced basal DALGreen autophagy-lysosome signal with retained rapamycin response, bafilomycin-dependent loss of group difference and explicit electrophysiology, microglia, behavior and mtDNA mutation-load limits in the 2026 organoid/co-culture study. The analysis is non-causal, non-pooled and hypothesis-generating.

Conclusions: Public POLG astrocyte-lineage evidence supports an assay-state interpretation rule: basal DALGreen signal, FCCP-induced mitophagy/protein state and reactive/co-culture endpoints should not be merged into a single rescue claim. This boundary is POLG- and astrocyte-lineage specific, but it does not establish neuronal functional recovery, patient benefit, variant-general rescue or clinical guidance.

Keywords: POLG; DNA polymerase gamma; astrocytes; mitophagy; autophagy flux; DALGreen; PINK1-Parkin; reactive astrocytes

1 Introduction

POLG encodes DNA polymerase gamma, the mitochondrial DNA polymerase catalytic subunit. POLG neurological disease models therefore begin with a gene-specific mtDNA-maintenance lesion, but astrocyte papers can still lose specificity if every mitochondrial, mitophagy and inflammatory readout is treated as one rescue axis. The present question asks whether public POLG iPSC-derived astrocyte, organoid and co-culture papers preserve an assay-state bottleneck that separates quality-control failure from reactive-astrocyte toxicity, or whether the readouts collapse into generic mitochondrial stress [1-3].

The strongest alternative is ordinary context dependence. Under that account, lower mitochondrial function, maturation differences, culture state, non-isogenic line structure and assay choice explain the observations; POLG status adds no separable discriminator once generic mitochondrial stress is acknowledged. The selected account predicts a different matrix under the same source conditions: FCCP/PINK1-Parkin/LC3-p62 induced mitophagy findings, basal DALGreen autophagy-lysosome signal and reactive/co-culture toxicity should remain non-equivalent endpoints.

This is not a general mitochondrial-disorder analysis. The core papers use POLG mutation carrier lines, including WS5A p.W748S/W748S, CP2A p.A467T/W748S and an Alpers-related p.A467T/p.P589L line in the 2026 organoid/co-culture work [1-3]. The analysis depends on POLG-linked mtDNA maintenance, human astrocyte-lineage differentiation, and the particular assay-state contrasts in these papers. Adjacent primary studies in POLG neural stem cells and neurons, astrocyte mtDNA-depletion animals and A1-like astrocyte induction help bound the interpretation, but they are not substituted for the POLG astrocyte source comparison [4-6].

2 Methods

Primary citation metadata and public records were verified on 11 October 2026 against publisher, PMC or PubMed records. The focal sources were Chen et al. 2021, Hong et al. 2024 and Yangzom et al. 2026 [1-3]. Current adjacent primary literature searches checked POLG iPSC neural-context work, astrocyte-specific mtDNA depletion and the A1-like reactive astrocyte source literature [4-6]. Complete normalized public texts for the three focal POLG papers were inspected and bound to SHA-256 source hashes during source review.

The public source-comparison pilot coded only explicit public source information: source identifier, model, genotype or line context, assay, perturbation state, reported endpoint and limitation count. The coding retained deposited measurement labels. FCCP mitophagy detection, PINK1/Parkin/LC3/p62 protein state, DALGreen fluorescence under basal, rapamycin and bafilomycin states, A1-like markers, cytokines, morphology and co-culture endpoints were not converted into absolute mitochondrial function, viability, membrane voltage, neuronal electrophysiology or clinical efficacy. Figure 1 summarizes the workflow from frozen question through source verification, coding, sensitivity checks and bounded synthesis.

Four sensitivity checks were declared. Assay-state separation was decision-changing because it determined whether one rescue label

was scientifically valid. Translation boundary was decision-changing because cell and organoid readouts cannot establish patient benefit. Same-lineage family was a robustness check because the focal sources share related POLG line families and laboratory lineage. Exact assay label was exploratory because harmonizing labels can change interpretation without adding biology. No pooling was performed because the sources are heterogeneous iPSC-derived astrocyte, organoid and co-culture studies rather than independent quantitative cohorts.

The public bundle includes manuscript source, assay-state matrix, evidence table, source-data JSON, analysis summary, analysis code, figures, figure geometry measurements, biological-audit supplements, QA record, Gemini review and checksums. A supplementary biological audit ledger records exact manuscript anchors, source identifiers, source excerpts, source-version hashes and unresolved assumptions. The ledger checks quotation and manuscript-location integrity only; it is not proof of biological truth, clinical efficacy or guideline suitability.

3 Results

3.1 Evidence synthesis

The source-comparison pilot coded 17 assay rows across three POLG astrocyte-lineage records. Chen et al. used iPSC-derived astrocytes from WS5A and CP2A POLG patient lines and reported downregulated mitophagy/autophagy-related genes, impaired FCCP-induced mitochondrial autophagosome formation, lower p62, reduced LC3B-II lipidation, lower LAMP2A and PINK1/Parkin pathway changes; NR plus metformin improved measured mitophagy machinery in that source context [1]. Hong et al. used related POLG astrocytes and co-culture systems and reported mitochondrial membrane-potential loss, energy failure, complex 1 and IV loss, NAD⁺/NADH disturbance, mtDNA depletion, A1-like reactive features and neuron-toxic co-culture effects [2]. Yangzom et al. used astrocytes, cortical organoids and co-culture systems and reported reduced basal DALGreen signal in POLG astrocytes, retained rapamycin induction, loss of group difference under bafilomycin states and NR-associated improvement in several inflammatory, mitochondrial and co-culture readouts [3].

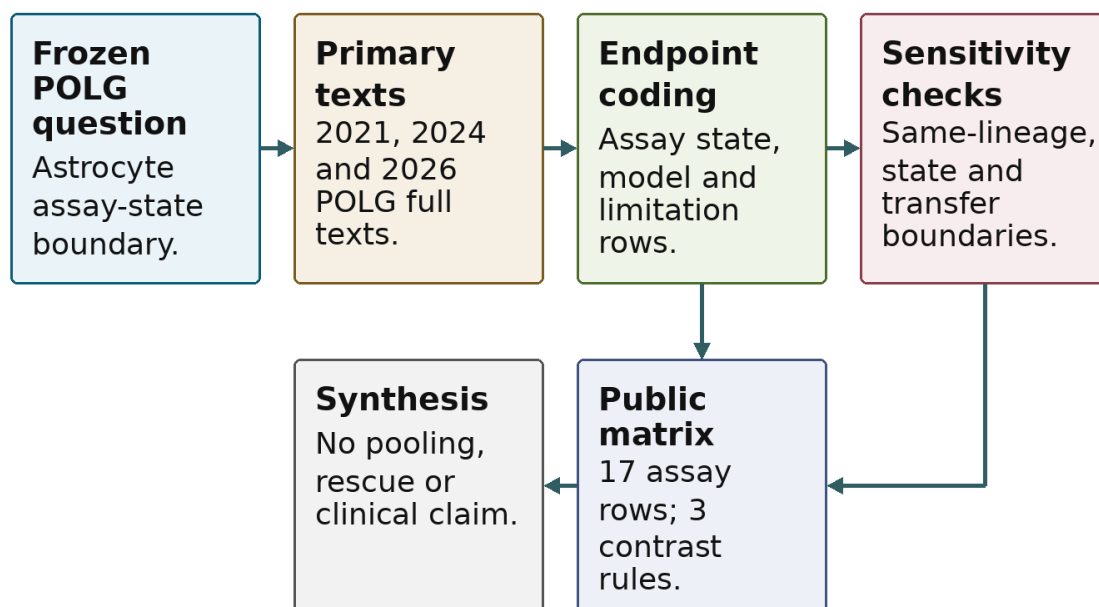
Table 1 preserves the central evidence and the interpretive limits. The table intentionally separates induced mitophagy/protein state, basal and perturbed autophagy-lysosome signal, reactive-state co-culture phenotypes and adjacent primary context.

The matrix supports an assay-state boundary rather than a single generic rescue label. The 2021 paper's FCCP-induced mitophagy and PINK1/Parkin/LC3/p62 measurements are stress-induced quality-control and protein-state measurements [1]. The 2026 paper's DALGreen assay is a fluorescent acidic autophagic-vesicle readout under basal, rapamycin and bafilomycin conditions, and the source itself leaves electrophysiology, behavior, microglia and mtDNA mutation-load stability unresolved [3]. The 2024 paper's A1-like and co-culture results concern reactive astrocyte phenotype and neuronal support or toxicity, not direct proof that the same mitophagy pathway state has been normalized [2]. Figure 2 shows the resulting concept boundary and the competing generic-stress alternative.

Table 1. Public POLG astrocyte assay-state evidence and interpretation limits.

ID	Model and perturbation	Key finding	Interpretive limitation
[1]	POLG iPSC-derived astrocytes from WS5A p.W748S/W748S and CP2A p.A467T/W748S lines plus controls.; FCCP-induced mitophagy assay, PINK1/Parkin and LC3/p62/LAMP2A protein measurements, NR plus metformin exposure.	Reported downregulation of mitophagy/autophagy-related genes and impaired stress-induced mitochondrial autophagosome formation; dual NR/metformin improved measured mitophagy machinery.	FCCP and protein-state readouts are induced quality-control measurements, not basal autophagy flux, neuron function, patient efficacy or a variant-general rescue rule.

ID	Model and perturbation	Key finding	Interpretive limitation
[2]	POLG iPSC-derived astrocytes and direct or indirect neuron co-cultures from WS5A and CP2A line families.; Mitochondrial function, NAD/NADH, mtDNA copy number, A1-like markers, proliferation, invasion and neuron co-culture assays.	Reported mitochondrial dysfunction, mtDNA depletion, disturbed NAD metabolism, A1-like reactive features and toxic effects on neurons in co-culture.	Reactive and co-culture endpoints remain distinct from mitophagy assays and do not isolate generic mitochondrial stress, maturation or shared-lineage structure.
[3]	POLG iPSC-derived astrocytes, cortical organoids and astrocyte-neuron co-cultures including WS5A, CP2A and Alpers-related variants.; DALGreen autophagic flux under basal, rapamycin and bafilomycin conditions with NR treatment and co-culture readouts.	Reported reduced basal DALGreen signal in POLG astrocytes, retained rapamycin induction, loss of group difference under bafilomycin states and NR-associated reductions in inflammatory/co-culture abnormalities.	DALGreen is an acidic autophagic-vesicle signal; electrophysiology, behavior, microglia-containing immune context and mtDNA mutation-load stability were not established.
[4]	POLG patient-derived iPSC neural stem cells and dopaminergic neurons from related disease modeling work.; Lineage maturation from iPSCs to neural stem cells and dopaminergic neurons.	Reported mtDNA depletion, complex 1 loss, increased reactive oxygen species, senescence and disturbed NAD metabolism in POLG neural models.	Neural precursor and neuron findings support lineage-specific context; they do not make astrocyte mitophagy, basal flux and reactivity interchangeable.
[5]	Mouse astrocyte-specific mtDNA depletion model through Twinkle inactivation.; Astrocyte mtDNA-loss model with brain pathology assessment.	Reported chronic astrocyte activation, microgliosis, secondary neurodegeneration and spongiotic encephalopathy after astrocytic mtDNA loss.	This animal mtDNA-depletion evidence supports astrocyte vulnerability but is not POLG iPSC genotype-specific and does not validate human rescue endpoints.
[6]	Purified mouse and human astrocyte systems plus in vivo injury and microglial activation contexts.; A1 reactive astrocyte induction by activated microglial factors including IL-1alpha, TNF and C1q.	Reported neurotoxic reactive astrocytes that lost several normal astrocyte functions and contributed to neuronal death in defined systems.	A1 terminology is a context marker; it should not be treated as proof that POLG astrocyte reactivity, mitophagy impairment and neuronal functional rescue are one process.



Public artifacts use declared literature rows and source hashes; administrative working files are excluded.

Figure 1. Public source-comparison workflow for the POLG astrocyte assay-state analysis. The workflow preserves the frozen POLG question, verifies complete public primary texts, codes assay state and model context, applies sensitivity checks and produces a bounded synthesis without patient-level or unpublished inputs or clinical efficacy inference.

3.2 Evidence-coded sensitivity analysis

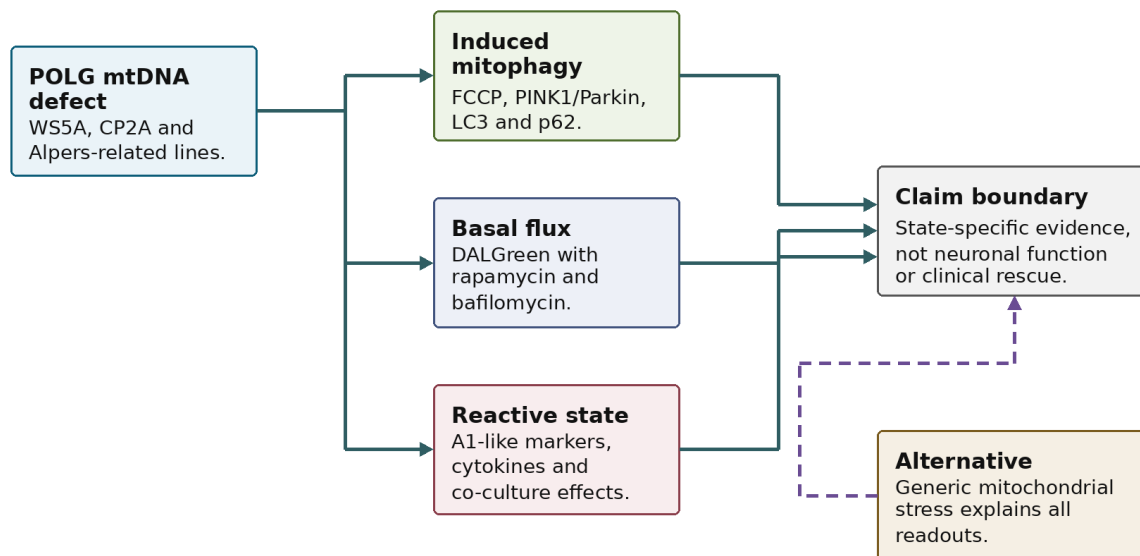
The decision-changing assay-state separation check retained three endpoint classes rather than one generic mitochondrial-stress row. The same-lineage robustness check retained line-family dependence as a limitation, so the matrix is not counted as independent biological replication across unrelated laboratories. The decision-changing translation-boundary check prevented mitochondrial, cytokine, morphology or organoid results from becoming neuronal electrophysiology, behavior or clinical-benefit claims. The exploratory exact-label check retained source measurement labels and did not convert DALGreen, FCCP mitophagy detection or co-culture morphology into absolute function.

Adjacent POLG iPSC neural work supports the tissue-specific caution. Liang et al. reported that patient-derived POLG neural stem cells and dopaminergic neurons recapitulated mtDNA

depletion, complex 1 loss, increased reactive oxygen species, senescence and disturbed NAD metabolism [4]. Mouse astrocyte-specific mtDNA depletion through Twinkle inactivation supports the plausibility that astrocyte mtDNA maintenance can drive brain pathology, but that animal model does not validate human POLG iPSC rescue endpoints [5]. Liddelow et al. defined an A1-like neurotoxic reactive astrocyte framework under activated microglial conditions, which helps interpret A1 terminology while also warning against treating marker labels as a full functional state in POLG cultures [6].

4 Discussion

The principal contribution is a measurement rule for POLG astrocyte interpretation. In the public source family, POLG-driven mtDNA and oxidative-phosphorylation defects should be analyzed through assay state: FCCP-induced mitophagy and



Solid connectors are source-coded relationships; dashed connector is the strongest alternative interpretation.

Figure 2. Concept summary for the POLG astrocyte assay-state boundary. POLG mtDNA defects feed induced mitophagy, basal DALGreen and reactive/co-culture readouts that converge on a bounded interpretation; the dashed connector shows the competing generic-stress account.

PINK1/Parkin/LC3/p62 protein findings, basal or perturbed DALGreen autophagy-lysosome signal, and reactive astrocyte/co-culture phenotypes are not equivalent endpoints. A treatment or perturbation can improve one of these readout classes without proving that every quality-control, inflammatory, neuronal or clinical endpoint has been rescued.

This contribution differs from the closest primary papers because it does not re-report a new wet-lab rescue. Chen et al. asked how POLG mutations affect mitophagy and how NR/metformin alters that pathway in iPSC-derived astrocytes [1]. Hong et al. asked how POLG astrocyte mitochondrial dysfunction relates to reactive phenotypes and neuron toxicity [2]. Yangzom et al. asked how NR affects glial inflammation, mitochondrial function and co-culture or organoid readouts [3]. The present analysis instead compares the assay states across those papers and identifies which claims remain separated under the same public source family.

The contribution is also distinct from the closest current Mito Map POLG portfolio manuscripts. The POLG dC/dT endpoint-retention paper asks whether public NMDS, GDF-15, EEG and quality-of-life endpoints remain concordant after deaths, withdrawals, missing biomarker windows and open-label single-arm design are preserved; this paper does not analyze dC/dT, clinical endpoints or trial-family retention. The POLG liver-vulnerability paper asks whether genotype, primary liver mtDNA maintenance, hepatic drug or injury stress and regeneration or proliferation endpoints discriminate liver failure mechanisms; this paper has no VPA, CCl₄, transplant-liver, primary liver mtDNA or regeneration endpoint. The POLG vascular conditioned-media paper asks whether donor-recipient transfer controls separate diffusible vascular smooth-muscle effects from cell-autonomous p53/calcification or PolG acetylation/senescence mechanisms; this paper has no vascular lineage, conditioned-media transfer, p53 calcification or K1039 acetylation analysis. The new result is therefore not a renamed POLG cautionary synthesis but a source-bound astrocyte-lineage assay-state rule built from Chen 2021, Hong 2024 and Yangzom 2026.

The practical research decision is concrete. Future POLG astrocyte rescue studies should pair basal autophagy-lysosome flux, induced mitophagy, lysosomal blockade, reactive-state markers, mitochondrial mass and neuron-function endpoints before describing a perturbation as a mitophagy or neuroglial rescue. Genotype line, isogenic-control status, maturation stage, mitochondrial mass, viability, lysosomal acidification, FCCP/rapamycin/bafilomycin treatment state, donor or clone

identity and co-culture duration should be reported together. If a same-line experiment showed that every readout scaled only with generic mitochondrial mass, viability or maturation markers, the POLG-specific assay-state interpretation would be weakened.

5 Limitations

The analysis is a public source-comparison pilot. It did not reanalyze raw images, raw sequencing, electrophysiology, behavior, patient outcomes, unpublished source archives or non-public case data. The focal studies share related line families and laboratory lineage, so they should not be treated as independent replication. Missing genotype transfer evidence remains bounded: the claim is not extended to all POLG variants, hepatocytes, vascular cells, fibroblast nucleoside rescue, purified POLG biochemistry or clinical treatment.

Several measurement limits remain material. DALGreen fluorescence is treated as the source-defined acidic

autophagic-vesicle signal, not absolute autophagic flux in every biological sense. Mean mitochondrial dye intensity, mtDNA copy-number assays, cytokines, A1-like markers and morphology each answer different questions. The 2026 source explicitly leaves electrophysiology, behavior, microglial immune context and mtDNA mutation-load deep sequencing unresolved [3]. The absence of a direct contradictory same-line POLG astrocyte replication study is an unresolved limitation rather than support.

6 Conclusions

Public POLG astrocyte-lineage papers support an assay-state boundary: induced mitophagy/protein-state assays, basal and perturbed DALGreen autophagy-lysosome signal and reactive/co-culture phenotypes should remain separate decision endpoints. The evidence supports a reproducible source-comparison rule, not clinical efficacy, patient advice or a variant-general rescue mechanism. Future experiments can test the rule directly by measuring basal flux, induced mitophagy, lysosomal blockade, reactive state and neuronal function in the same genotype-controlled samples.

Declarations

Data and code availability. The repository deposit includes the manuscript source, evidence table, source data, analysis code, figures, measured figure geometry, biological-audit supplements, layout reports, QA record, Gemini review and checksums needed to

replay the table, counts, figures and artifacts. Private replay inputs are retained locally and excluded from public manifests.

Source verification. The biological audit is a manuscript-integrity ledger, not proof of biological truth or clinical efficacy. Corpus anchors and archived normalized source files were replayed against recorded hashes; UNKNOWN or NOT_RUN biological interpretation statuses remain visible.

Ethics statement. This literature synthesis used no identifiable patient data and required no new human-participant or animal research.

Funding. No external funding was reported for this working paper.

Competing interests. The author reports no competing interests for this working paper.

Author contributions. Andrew Friedl conceived the project, directed the analysis, reviewed the evidence package, and approved the manuscript for repository review.

AI assistance. AI tools assisted literature synthesis, drafting, data visualization, formatting and QA preparation. Gemini independently reviewed the exact rendered PDF when artifact-bound review was available. Andrew Friedl remains solely responsible for the content and is the sole author.

References

- [1] Chen A, Kristiansen CK, Hong Y, Kianian A, Fang EF, Sullivan GJ, Wang J, Li X, Bindoff LA, Liang KX. Nicotinamide Riboside and Metformin Ameliorate Mitophagy Defect in Induced Pluripotent Stem Cell-Derived Astrocytes With POLG Mutations. *Front Cell Dev Biol.* 2021;9:737304. doi:10.3389/fcell.2021.737304. PMID:34631714.
- [2] Liang KX, Kianian A, Chen A, Kristiansen CK, Hong Y, Furriol J, Soleimani M, Sulheim D, Miletic H, Tzoulis C, Bindoff LA. Activation of Neurotoxic Astrocytes Due to Mitochondrial Dysfunction Triggered by POLG Mutation. *Int J Biol Sci.* 2024;20(7):2860-2880. doi:10.7150/ijbs.93445. PMID:38904024.
- [3] Yangzom T, Chen A, Lundberg BC, Liang KX. Nicotinamide riboside reduces glial inflammation and boosts mitochondrial function. *Int J Biol Sci.* 2026;22(11):6035-6063. doi:10.7150/ijbs.119262. PMID:42328457.
- [4] Liang KX, Kristiansen CK, Mostafavi S, Vatne GH, Zantigh GA, Kianian A, et al. Disease-specific phenotypes in iPSC-derived neural stem cells with POLG mutations. *EMBO Mol Med.* 2020;12(10):e12146. doi:10.15252/emmm.202012146. PMID:32840960.
- [5] Ignatenko O, Chilov D, Paetau I, de Miguel E, Jackson CB, Capin G, Paetau A, Terzioglu M, Euro L, Suomalainen A. Loss of mtDNA activates astrocytes and leads to spongiform encephalopathy. *Nat Commun.* 2018;9(1):70. doi:10.1038/s41467-017-01859-9.
- [6] Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Munch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature.* 2017;541(7638):481-487. doi:10.1038/nature21029. PMID:28099414.