

POLG Variant Conditioned-Media Effects Define a Transfer-Control Boundary in Vascular Smooth Muscle

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Abstract

Background: POLG encodes mitochondrial DNA polymerase gamma's catalytic subunit. Vascular-cell POLG papers now include conditioned-media transfer, PolG/p53 calcification and PolG acetylation/senescence evidence, leaving unresolved whether vascular phenotypes reflect diffusible donor-cell effects, ordinary cell-autonomous dysfunction, or separate p53 and K1039 mechanisms.

Objective: The selected question asks whether same-condition transfer experiments distinguish a diffusible POLG-loss mitogenic consequence from cell-autonomous mitochondrial dysfunction, p53-linked calcification or PolG acetylation/senescence.

Methods: Complete public primary texts archived from PubMed Central and publisher pages were checked on 2026-10-12 for Rothwell 2025, Wang 2022 and Wang 2026. A current primary-literature search identified a 2025 ATVB POLG/PARP1 vascular-calcification lead, but complete text was not archived in this bundle and detailed claims from that record were held as unresolved. Three complete primary records were coded for model, perturbation, lineage, transfer design, endpoint class, observed evidence, limitations and same-condition discriminability. The analysis was a bounded public source-coded reanalysis and transfer-control synthesis; no raw-image reanalysis, pooled effect size, patient-level inference, secreted-factor identity, clinical efficacy or universal POLG rescue mechanism was inferred.

Results: One complete primary source directly tested a transfer condition: POLG variant-transfected HeLa donor media reduced naive-culture doubling times, while A7r5 donor cultures showed POLG variant mtDNA-copy-number and growth effects with modest same-condition TMRM, MitoSOX and Seahorse changes [1]. Two complete comparator sources supported cell-autonomous vascular PolG accounts without donor-recipient transfer: PolG D257A/D257A aggravated calcification while wild-type PolG supported p53 recruitment and mitochondrial-function rescue [2], and PolG K1039 acetylation altered mtDNA binding, HASMC contractility, hyperproliferation, mitochondrial dysfunction and senescence [3]. Removing the transfer source changed the contribution from a donor-recipient boundary rule to a general vascular PolG synthesis; removing either comparator left a substantive cell-autonomous alternative.

Conclusions: Current complete-text evidence supports a transfer-control rule rather than a new biological validation claim. POLG vascular remodeling claims require donor-recipient conditioned-media controls, donor-cell stress controls, serum/glucose matching and parallel cell-autonomous mtDNA, respiration, calcification and senescence endpoints before being interpreted as purely cell-autonomous mtDNA depletion, p53/calcification or PolG acetylation/senescence. The same analysis cannot be applied unchanged to any mitochondrial disorder because the selected discrimination depends on POLG variant identity, PolG D257A and K1039 contexts, polymerase-gamma function and vascular-cell transfer evidence.

Keywords: POLG; DNA polymerase gamma; vascular smooth muscle; conditioned media; mitochondrial DNA; calcification; senescence

1 Introduction

POLG has a specific role in mitochondrial DNA replication and genome maintenance as the catalytic subunit of DNA polymerase gamma. Vascular-cell studies that name PolG are therefore not interchangeable with generic mitochondrial stress studies. A POLG variant, a D257A exonuclease-deficient model, a K1039 acetylation state and a PARP1/POLG PARylation lead can all involve mitochondrial dysfunction while posing different causal questions.

The selected question asks whether POLG-loss vascular smooth-muscle and comparator vascular-cell papers require same-condition transfer experiments before assigning vascular phenotypes to cell-autonomous dysfunction. This question is narrower than a review of POLG disease biology. It asks whether donor-cell POLG perturbation, recipient-cell growth after conditioned media, mtDNA-copy-number movement, p53-linked calcification and K1039 acetylation/senescence can be separated in public primary sources.

The strongest competing account is ordinary cell autonomy. Under this account, POLG-related vascular phenotypes arise from mtDNA damage, mitochondrial-function loss, p53-linked calcification or PolG acetylation state, and any conditioned-media response reflects transfection burden, culture stress, serum or glucose context, or nonspecific mitochondrial injury rather than a separable transferable POLG-loss signal. The selected concept predicts that recipient growth after conditioned-media transfer remains an explicit discriminator because it can occur without recipient POLG variant expression.

2 Methods

The reviewed candidate-selection dossier, archived public full-text sources and current primary-literature searches were checked on 2026-10-12. Complete archived texts were available for three central primary records: Rothwell 2025, Wang 2022 and Wang 2026 [1-3]. A 2025 ATVB paper on PARP1-mediated POLG PARylation in vascular calcification was identified in current indexed search and is listed as a screened lead [4]. Because complete text for that record was not archived for this bundle, it was not used as central evidence for detailed methods, endpoint timing or mechanistic claims.

Eligibility required a public primary source directly concerning POLG or PolG in vascular smooth-muscle or vascular-cell systems, with at least one endpoint relevant to donor-recipient transfer, mtDNA copy number, mitochondrial function, calcification, p53 recruitment, acetylation, contractility, proliferation or senescence. Abstract-only and review-only sources were not used as central complete-text evidence. Internal source identifiers and candidate dossiers were not used as citation metadata.

A machine-readable evidence and assumption ledger is provided in Supplementary File S1, with a readable companion in Supplementary File S2. The ledger records exact manuscript anchors, source identifiers, evidence excerpts, source-version hashes, actual check outcomes and unresolved assumptions; it is not a proof of biological truth, clinical validity or therapeutic efficacy.

Each primary source was coded for genotype or PolG perturbation, biological system, transfer-test status, cell-autonomous readouts, recipient readouts, observed evidence, interpretation limit and role in the competing-model analysis. The primary endpoint was source-level presence of a same-condition donor-recipient transfer discriminator versus cell-autonomous vascular PolG mechanisms.

No pooled effect size was estimated. The sources differ by species, cell lineage, POLG variant or mutation, culture condition, perturbation duration, endpoint and availability of recipient-cell transfer. The analysis therefore measured information gain: whether a source changes the next experimental decision about transfer controls versus p53/calcification, K1039 acetylation/senescence or

nonspecific mitochondrial-stress controls.

3 Results

The evidence step first separates the only source-coded transfer assay from the cell-autonomous comparator papers, then tests whether removing any source changes the research decision.

3.1 Evidence synthesis

Transfer coding identifies one donor-recipient source. Rothwell 2025 is the only complete-text source in the central set with a direct conditioned-media transfer test. The study expressed POLG variants p.Tyr955Cys, p.Arg964Cys, p.Asn1098Ile and p.Arg1138Cys, measured mtDNA copy-number readouts by imaging and ddPCR, assessed TMRM, MitoSOX and oxygen consumption, and reported reduced doubling times in naive cultures exposed to conditioned media from variant-transfected HeLa cells [1]. The donor-recipient result makes transfer a concrete experimental axis rather than a metaphor.

The same source does not identify the secreted factor and does not prove patient hypertension causality. HeLa recipient evidence is an assay tool, not a vascular-tissue claim. The useful result is the boundary: a recipient growth effect can be measured separately from donor-cell POLG variant expression and from donor-cell mitochondrial readouts.

Cell-autonomous vascular PolG comparators remain credible. Wang 2022 supplies a strong cell-autonomous vascular calcification comparator. In PolG D257A/D257A and vascular smooth-muscle calcification models, the paper linked PolG and p53 interaction to mitochondrial-function recovery and calcification resistance [2]. This supports a mechanism in which PolG status changes the vascular cell's own mitochondrial and calcification state without requiring donor-recipient media transfer.

Wang 2026 supplies a second cell-autonomous comparator in human aortic smooth muscle cell and POLG D257A/D257A contexts. The source links PolG K1039 acetylation to mtDNA binding, mitochondrial homeostasis, contractility, hyperproliferation and senescence [3]. This mechanism is POLG-specific, but it differs from a secreted mitogenic factor. It also means that POLG-loss phenotypes cannot be assigned to one universal rescue axis across catalytic, exonuclease, abundance, interaction, post-translational and transfer contexts.

Current literature expands the comparator space but not the transfer evidence. The current search identified Yang 2025, an ATVB primary record on PARP1-mediated POLG PARylation, ferroptosis-induced vascular calcification and Adora2a/Rap1 signaling [4]. The indexed abstract and citation metadata indicate another vascular PolG/calcification mechanism, but complete text was not archived in this bundle. It therefore functions as a current literature lead and not as a central detailed evidence source.

This limitation matters. The paper's existence makes the vascular PolG comparator space broader, but without complete text it cannot be used here to claim method details, endpoint timing, control adequacy or independence from Wang 2022 and Wang 2026. The claim depending on complete text remains held.

3.2 Evidence-coded sensitivity analysis

Three sensitivity checks were prespecified and labeled by role. The decision-changing check removed Rothwell 2025. Without that transfer-test source, the analysis no longer supports a donor-recipient boundary and becomes a general synthesis of cell-autonomous vascular PolG mechanisms. The robustness check removed Wang 2022. The p53/calcification alternative weakens, but K1039 acetylation and senescence still block a single transfer explanation. The exploratory check added the current Yang 2025 POLG/PARP1 lead. It strengthens the need to keep vascular PolG

Table 1. Primary-source evidence matrix for POLG vascular transfer and cell-autonomous comparator mechanisms. Abbreviations: HASMC, human aortic smooth muscle cell; mtDNA, mitochondrial DNA; PolG, DNA polymerase gamma; VSMC, vascular smooth muscle cell.

ID	Model and perturbation	Key finding	Interpretive limitation
[1]	A7r5 POLG-variant donor cultures; HeLa conditioned-media recipient assay.; Tyr955Cys, Arg964Cys, Asn1098Ile or Arg1138Cys; serum/glucose, inhibitor and media-transfer contexts.	POLG variants reduced mtDNA copy number; variant-transfected HeLa media reduced naive-culture doubling time.	Secreted factor not identified; HeLa recipient results are assay evidence, not direct human vascular disease evidence.
[2]	PolG D257A/D257A mice and vascular smooth-muscle calcification models.; Vitamin D3 or beta-glycerophosphate calcification; PolG/p53 transfection and mitochondrial assays.	D257A mice had more severe calcification; wild-type PolG supported p53 recruitment and mitochondrial-function rescue.	No donor-recipient conditioned-media transfer or recipient-proliferation control.
[3]	HASMC and POLG D257A/D257A vascular aging/senescence models.; K1039 acetylation state, Sirt3/GCN5 context, PolG knockdown/rescue and D257A background.	K1039 acetylation disrupted PolG-mtDNA binding and promoted HASMC hyperproliferation/senescence phenotypes.	No conditioned-media recipient growth test or secreted mitogenic mediator identification.
[4]	PubMed-indexed vascular smooth-muscle calcification/ferroptosis record; complete text not archived.; PARP1-mediated POLG PARYlation and Adora2a/Rap1 signaling from indexed abstract metadata.	Indexed abstract reports PARP1/PARYlation increase in calcification and mitigation by PARP1 knockdown.	Complete text not retrieved; detailed method or result claims remain a literature lead.

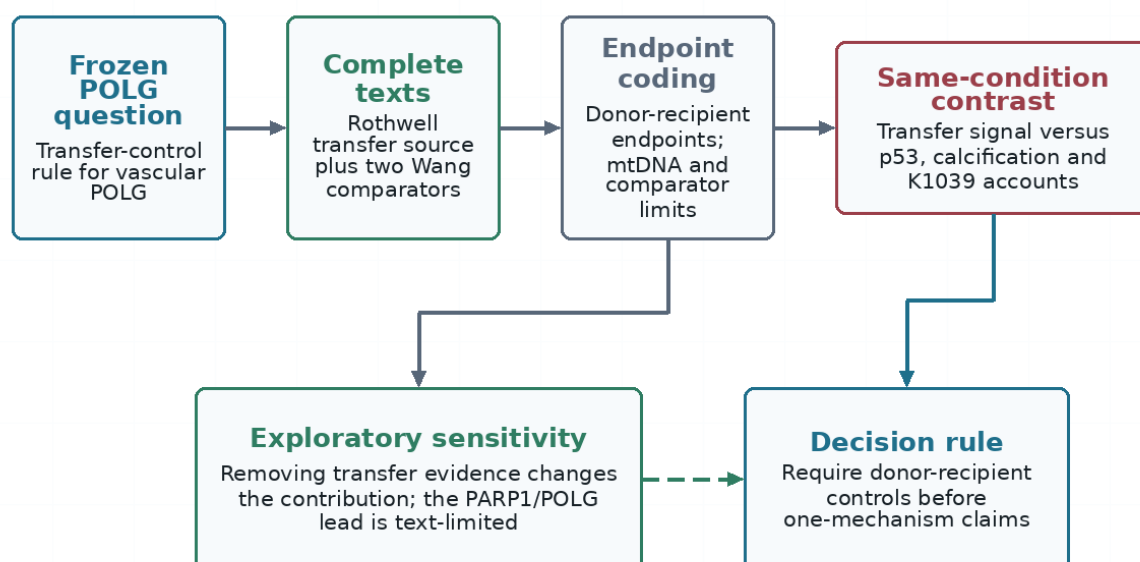


Figure 1. Source-bound workflow for the POLG vascular transfer-control analysis. The workflow preserves the frozen question, verifies complete primary texts, codes transfer and cell-autonomous endpoints, runs sensitivity checks and produces a donor-recipient decision rule.

mechanisms separate, but complete-text inaccessibility prevents using it as central evidence.

The sensitivity results support the same research decision: future vascular POLG work should prioritize matched conditioned-media transfer, donor-cell stress controls, serum/glucose matching and parallel cell-autonomous endpoints before interpreting mtDNA copy number, calcification, senescence or proliferation as one mechanism.

4 Discussion

The central contribution is not the discovery of a secreted factor. The contribution is a POLG-specific interpretation boundary. A subset of POLG variant-expressing donor cells can show mtDNA-copy-number changes and generate media that changes naive recipient growth in one complete-text source [1]. Nearby vascular PolG papers support p53/calcification and K1039 acetylation/senescence mechanisms without the same transfer test [2,3]. Those facts make transfer controls decision-critical.

This analysis would not be the same in any mitochondrial disorder. The source set depends on named POLG variants, PolG D257A, K1039 acetylation, mitochondrial DNA polymerase gamma function, and vascular-cell endpoints. A generic mitochondrial stress study could test conditioned media, but it would not answer whether POLG variant, exonuclease, p53-recruitment or PolG acetylation contexts should be interpreted as donor-recipient transfer versus cell-autonomous vascular mechanisms.

The negative result is also useful. If matched vascular smooth-muscle recipient assays show no growth effect after controlling donor-cell density, transfection burden, serum/glucose state and mitochondrial stress, the field should move toward cell-autonomous p53/calcification, K1039 acetylation/senescence or other PolG-intrinsic mechanisms. If the transfer effect persists, fractionation and rescue studies become higher priority than treating mtDNA copy number alone as the explanatory endpoint.

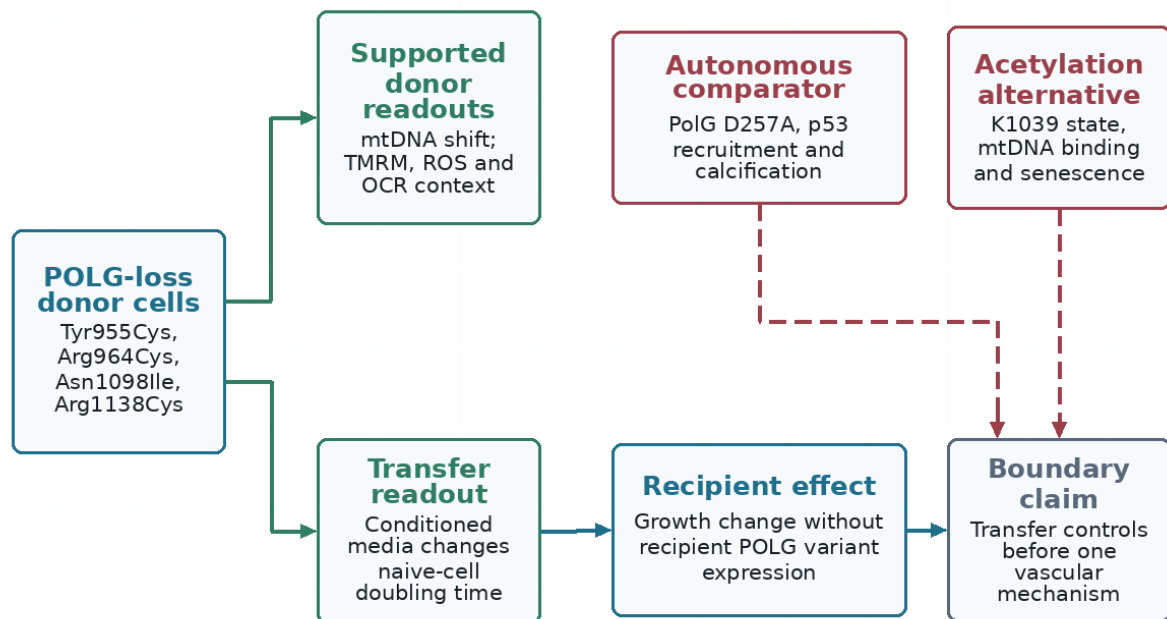


Figure 2. Concept map separating POLG-loss conditioned-media transfer evidence from cell-autonomous PolG vascular alternatives. Solid arrows show source-supported transfer and donor-cell readout relationships; dashed arrows mark comparator mechanisms that can explain vascular phenotypes without a media-transfer step.

5 Limitations

This working paper is a source-coded reanalysis and measurement-rule exercise. It does not reanalyze raw microscopy images, well-level time courses, mouse-level calcification data, mass-spectrometry files, patient-level hypertension data or secreted-factor fractions. Source-level coding can demonstrate discriminability and missing controls, not biological validation.

The source set is small because POLG-specific vascular transfer evidence is sparse. Complete text was available for three central primary sources. The current Yang 2025 POLG/PARP1 vascular-calcification lead was identified but not used centrally because complete text was not archived for this package [4]. Absence of a complete-text contradiction is not evidence that the transfer concept is true.

Translation limits are explicit. The selected concept does not infer hypertension treatment, patient vascular remodeling causality, clinical efficacy or the identity of a secreted mediator. HeLa conditioned-media recipient data are assay evidence only. Variant effects are not assumed to be interchangeable across p.Tyr955Cys, p.Arg964Cys, p.Asn1098Ile, p.Arg1138Cys, PolG D257A, K1039 acetylation or PARP1/POLG PARylation contexts.

6 Conclusions

Complete public primary evidence supports a conservative POLG-specific rule: vascular POLG remodeling claims require donor-recipient transfer controls before being interpreted as purely cell-autonomous mtDNA depletion, respiration failure, p53-linked calcification or PolG acetylation/senescence. The rule is ready for public source-coded analysis, but not for clinical guidance or a claim that the diffusible mediator has been identified.

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.

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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. Andrew Friedl remains solely responsible for the content and is the sole author.

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