

DOES RUBELLA CAUSE AUTISM?

*A Testable Endolysosomal Mechanism Linking RUBV P150-ARL8 Trafficking to Cholesterol
Unavailability and Developmental Regression*

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AUTHOR'S NOTE

Jacob would like to give credit to God for his amazing abilities.

MMR and Timing Integrated

ABSTRACT

We propose that endolysosomal cholesterol unavailability defines a mechanistic subtype of autism, particularly autism with developmental regression, and that rubella virus (RUBV) can act as an upstream cause of this state.

Persistent RUBV infection reproducibly impairs host cargo handling and alters membrane composition in independent human-cell systems. RUBV reorganizes cargo-accessible endolysosomal membranes into replication compartments, and the viral replicase P150 directly interacts with ARL8A. Independently, ARL8/HOPS controls CI-M6PR recycling, NPC2 lysosomal delivery, lysosomal free-cholesterol egress, and downstream endoplasmic-reticulum cholesterol availability.

A second experimentally established system - CLN8-CLN5-BMP - controls lysosomal membrane chemistry relevant to NPC2-mediated cholesterol transfer. Human autism independently exhibits abnormalities involving endolysosomal cargo processing and BMP, sphingomyelin, ceramide, sterol, cholesterol, lipoprotein, and membrane-lipid biology. Autism with developmental regression independently demonstrates a coordinated CNS sphingolipid phenotype.

These findings place RUBV upstream and human autism downstream of the same experimentally defined endolysosomal cholesterol-availability system. We therefore propose that they represent opposite ends of one causal pathway: RUBV-driven disruption of endolysosomal trafficking produces functional cholesterol unavailability, and this state contributes to a biologically defined form of autism, particularly autism with developmental regression.

Because rubella vaccine virus is ordinarily administered to children as part of MMR, the vaccine context cannot be excluded from the causal question. Large epidemiological studies have not found increased autism risk following MMR. Any extension of this RUBV mechanism to MMR must therefore survive direct vaccine-strain testing, population evidence, and temporal ordering.

Three tests are decisive: the RUBV mechanism, the compartmental cholesterol phenotype in autism with developmental regression, and the required temporal ordering between exposure, molecular state, and developmental loss.

1. THE CLAIM

The missing evidence is not whether RUBV disrupts host trafficking, whether autism involves endolysosomal and lipid biology, or whether ARL8/HOPS-NPC2 controls cholesterol

availability. Each is independently demonstrated.

The unresolved question is whether these established observations form the causal sequence proposed here.

We propose that RUBV can drive an endolysosomal trafficking state that produces functional cholesterol unavailability, and that this state contributes to a biologically defined form of autism, particularly autism with developmental regression.

This is not a claim that RUBV causes all autism. It is a mechanistically defined causal hypothesis for a biological subtype.

2. RUBV ALREADY PRODUCES THE PREDICTED UPSTREAM BIOLOGY

Persistent RUBV infection produces reproducible host cargo-handling dysfunction in human cells. In persistently infected human embryonic mesenchymal cells, surface EGF binding is increased while receptor-bound EGF internalization is profoundly impaired. In an independent persistent-RUBV system using human retinal pigment epithelial cells, particle attachment is preserved while phagocytic uptake is markedly reduced.

The retinal pigment epithelial system also exhibits altered membrane fatty-acid composition. Separately, RUBV reorganizes cargo-accessible endolysosomal membranes into replication structures. Endocytosed cargo reaches these virus-modified compartments, and P150 occupies the remodeled membrane system. RUBV therefore independently produces the trafficking phenotype, membrane phenotype, and organelle phenotype expected immediately upstream of the proposed cholesterol-availability mechanism.

3. P150-ARL8 PROVIDES A SPECIFIC MOLECULAR ENTRY POINT

The N-terminal region of RUBV P150 directly interacts with ARL8A, placing a viral replicase protein directly on a lysosome-associated trafficking GTPase.

Independent host experiments show that disruption of BORC-ARL8-HOPS impairs endosome-to-lysosome trafficking, disrupts CI-M6PR recycling, decreases lysosomal NPC2 association, increases NPC2 secretion, causes free cholesterol to accumulate in LAMP-positive compartments, reduces downstream cholesterol esterification/storage, and activates an ER cholesterol-starvation response.

ARL8/HOPS -> CI-M6PR -> NPC2 -> lysosomal cholesterol egress -> ER cholesterol availability.

This establishes the biological possibility central to the model: cholesterol can be intracellularly present while

functionally unavailable downstream.

4. AUTISM IS ALREADY IN THE PREDICTED SYSTEM

The human evidence does not require autism to be pulled toward this model by inference alone.

Human autism brain already demonstrates pathology within the endosomal-autophagic-lysosomal continuum.

Postmortem studies identify abnormal intracellular cargo involving endosomal, autophagic, and lysosomal compartments.

Human autism already demonstrates abnormalities in the membrane chemistry relevant to this compartment.

Independent developmental metabolomics identifies BMP, sphingomyelin, and ceramide among the dysregulated lipid classes.

Human autism repeatedly intersects sterol, cholesterol, lipoprotein, and membrane-lipid biology. Individual systemic directions vary across cohorts, but this model predicts intracellular partitioning and availability rather than a universal serum-cholesterol direction.

The missing evidence is not whether autism intersects endolysosomal and lipid biology. It does.

The missing evidence is whether the abnormalities already observed in autism resolve to the specific compartmental cholesterol-availability defect predicted here.

5. DEVELOPMENTAL REGRESSION IS THE SHARPEST HUMAN TEST

Autism with developmental regression provides the strongest current human phenotype for this model because the abnormality has been demonstrated in the CNS itself.

Direct CNS metabolomics identifies a coordinated sphingolipid state characterized by decreased sphingomyelin and increased ceramide, hexosylceramide, S1P/sphingosine, and sulfatides. Earlier regression-defined studies independently identify membrane-lipid remodeling.

These measurements do not establish NPC2 dysfunction or lysosomal cholesterol trapping. They do establish that developmental regression in autism is accompanied by measurable lipid-system biology in the affected neurological compartment.

The model predicts that this CNS lipid phenotype accompanies a deeper compartmental state in which lysosomal cholesterol mobilization and downstream availability are abnormal.

6. THE MEMBRANE CONTROL SYSTEM IS EXPERIMENTALLY DEFINED

NPC2-mediated cholesterol transfer depends on its membrane environment. BMP promotes NPC2-mediated

cholesterol transfer; sphingomyelin inhibits it; and ceramide can promote transfer and membrane interactions. CLN8 generates a stereospecific lysophosphatidylglycerol precursor used by CLN5 to produce BMP. Loss of CLN8 depletes relevant LPG/BMP species, while replacement of the missing product restores BMP synthesis. CLN8 also participates with CLN6 in lysosomal-enzyme cargo delivery, while CLN5 participates in retromer/CI-M6PR trafficking.

CLN8 -> LPG -> CLN5 -> BMP/LBPA -> NPC2-mediated cholesterol transfer.

Cargo delivery and lysosomal membrane chemistry therefore converge mechanistically on cholesterol availability.

7. THE TWO SIDES MEET

The RUBV and autism observations were generated independently, in different laboratories, eras, experimental systems, and human cohorts. Yet they converge on the same biological architecture. RUBV enters from upstream through persistent cargo-trafficking dysfunction, membrane remodeling, virus-modified endolysosomal compartments, and direct P150-ARL8A interaction.

Autism enters from downstream through abnormal endolysosomal cargo processing, BMP/SM/Cer dysregulation, sterol/cholesterol abnormalities, and a pronounced regression-associated CNS sphingolipid phenotype.

Between them lies a directly demonstrated host pathway - ARL8/HOPS -> CI-M6PR -> NPC2 -> lysosomal cholesterol egress -> ER cholesterol availability - and a directly demonstrated membrane-control system - CLN8 -> CLN5 -> BMP -> NPC2.

We therefore propose that these are opposite ends of one causal pathway: RUBV-driven disruption of endolysosomal trafficking produces functional cholesterol unavailability, and this state contributes to a biologically defined form of autism, particularly autism with developmental regression.

8. MMR CANNOT BE LEFT OUT OF THIS QUESTION

For most vaccinated children, exposure to a rubella vaccine virus does not occur in isolation; it occurs through the combined measles-mumps-rubella vaccine. Any proposed RUBV-to-autism mechanism therefore has to confront MMR directly.

The present model focuses on RUBV because the molecular evidence assembled here concerns rubella biology - P150, ARL8, endolysosomal remodeling, and the downstream cholesterol-availability pathway. The evidence

does not permit effects of the rubella component to be assigned automatically to measles, mumps, or the combined formulation.

Large population studies have not detected an increased autism risk following MMR vaccination. Studies examining developmental regression and post-vaccination timing have likewise not established temporal clustering sufficient to support MMR as a common population-level cause of autism.

Those findings are a major constraint on any MMR-related causal hypothesis. A subgroup hypothesis does not overturn null population evidence. It creates a harder requirement: the subgroup must be prospectively identifiable and the predicted mechanism must be demonstrated directly rather than inferred after diagnosis.

9. THE RUBELLA COMPONENT OF MMR MUST BE TESTED DIRECTLY

Wild-type RUBV infection and live-attenuated rubella vaccination are not interchangeable experimental exposures.

Demonstrating P150-ARL8-HOPS-NPC2 disruption during wild-type RUBV infection would not establish that the attenuated rubella component of MMR produces the same cellular state.

If the rubella component of MMR participates in the mechanism proposed here, vaccine-strain RUBV itself must reproduce the relevant molecular sequence: P150-ARL8 engagement, altered HOPS/CI-M6PR/NPC2 trafficking, impaired compartmental cholesterol availability, and the predicted downstream lipid state.

The direct experiment is unavoidable: compare wild-type RUBV and the relevant attenuated rubella vaccine strain head-to-head through the complete pathway. If the vaccine strain does not generate the predicted state, the MMR extension loses support. If it does, population-level null findings still require direct identification of the proposed susceptible subgroup.

10. TIMING IS A DECISIVE TEST, NOT A DETAIL

Any RUBV/MMR-to-autism causal model must survive chronology.

MMR is commonly administered during the developmental period in which autism-related differences and developmental regression may become clinically apparent. Temporal proximity alone therefore cannot establish causation.

Population studies have not demonstrated a reproducible clustering of autism or developmental regression after MMR. This weighs strongly against a simple model in which MMR commonly and rapidly produces

autism.

The present model makes a harder temporal prediction. Exposure must precede the molecular lesion. The molecular lesion must precede or accompany the downstream lipid state. And the downstream biological state must precede or accompany developmental loss.

EXPOSURE -> P150/replication-compartment maturation -> ARL8/HOPS perturbation ->

CI-M6PR/NPC2/BMP/FC phenotype -> membrane/sphingolipid remodeling -> developmental regression.

If the predicted cholesterol/BMP/NPC2 phenotype is demonstrably present before the proposed exposure, that exposure cannot explain it. If the phenotype appears only after developmental regression, it may represent consequence rather than cause. If no reproducible temporal ordering exists, the causal model weakens. Timing therefore does not rescue the model. It is one of the ways the model can fail.

11. THREE TESTS NOW DECIDE THE MODEL

CAUSAL TEST 1 - RUBV MECHANISM. Perturb the P150-ARL8 interaction while preserving
relevant

P150 functions. Measure ARL8/HOPS association, VPS39/VPS41 recruitment, CI-M6PR recycling, NPC2 lysosomal localization/secretion, lysosomal free cholesterol, cholesterol esterification, and ER sterol signaling.

CAUSAL TEST 2 - HUMAN AUTISM. Compare carefully phenotyped autism with
developmental

regression, autism without developmental regression, and unaffected controls. Measure GPG, LPG, species-resolved BMP/LBPA, NPC2 localization/function, lysosomal free cholesterol, and LDL-derived cholesterol delivery to the ER.

CAUSAL TEST 3 - TEMPORAL ORDERING. Demonstrate that the predicted molecular state
arises after the

proposed exposure and before or during developmental loss. For any MMR extension, directly test the relevant attenuated rubella vaccine strain and reconcile the result with existing population-level evidence.

Failure at any of these disease-specific tests requires the corresponding part of the causal model to be revised or rejected.

THE COMPLETE MODEL

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WILD-TYPE RUBV
OR
RUBELLA COMPONENT OF MMR*
|
v
RUBV P150
|
direct interaction
v
ARL8A
|
CAUSAL TEST #1
|
v
ARL8 / HOPS
VPS39 / VPS41
|
+-----+-----+
|               |               |
v               v               v
CARGO AVAILABILITY      MEMBRANE CHEMISTRY
|               |               |
CI-M6PR / IGF2R      CLN8 -> LPG -> CLN5
|               |               |
v               v               v
NPC2               BMP/LBPA
|               |               |
+-----+-----+
|               |               |
v               v               v
NPC2-MEDIATED CHOLESTEROL TRANSFER
|               |               |
v               v               v
LYSOSOMAL FC EGRESS
|               |               |
v               v               v
ER CHOLESTEROL AVAILABILITY
|               |               |
v               v               v
MEMBRANE / SPHINGOLIPID HOMEOSTASIS
|
CAUSAL TEST #2
|
v
AUTISM, PARTICULARLY
DEVELOPMENTAL REGRESSION

CAUSAL TEST #3:
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THE ENTIRE SEQUENCE MUST OCCUR
IN THE REQUIRED TEMPORAL ORDER.

*The vaccine-strain extension requires direct testing;
wild-type RUBV data do not establish it.

13. CONCLUSION

The literature now supports more than a generic association between RUBV, lipids, and autism. It supports a specific causal model in which an experimentally demonstrated RUBV trafficking phenotype enters a host pathway controlling lysosomal cholesterol availability, while human autism independently exhibits abnormalities in the downstream organelle, lipid, and cholesterol systems governed by that pathway. Autism with developmental regression provides the strongest candidate phenotype because its CNS lipid signature lies directly within this downstream biological space.

We propose that RUBV-driven endolysosomal trafficking dysfunction produces functional cholesterol unavailability, which contributes to a biologically defined form of autism and is particularly relevant to developmental regression.

MMR must be confronted because rubella vaccine virus is commonly administered in that formulation. Existing population evidence weighs strongly against MMR as a common cause of autism. Accordingly, any extension of this model to MMR must demonstrate the pathway with the vaccine strain itself, identify the proposed susceptible biology directly, and satisfy temporal ordering.

Three tests separate the causal model from demonstration: establish the P150-ARL8-HOPS-CI-M6PR-NPC2 mechanism during RUBV infection; establish the predicted compartmental cholesterol-unavailability phenotype in autism with developmental regression; and establish the required chronology between exposure, molecular state, and developmental loss.

Until those links are demonstrated, RUBV causation of autism through this pathway remains a causal hypothesis - not an established conclusion.

But the hypothesis is now required to survive mechanism, human autism biology, MMR epidemiology, vaccine-strain testing, and chronology. Its upstream viral phenotype, downstream human-autism phenotype, and intervening host machinery are independently experimentally anchored, leaving three explicit and falsifiable disease-specific tests.

SEPTEMBER 27, 2026 SYNCHRONIZATION ADDENDUM

Paper VI remains the disease-level causal-test paper. Its central boundaries remain controlling: the model concerns a proposed biological subtype rather than autism generally; MMR population evidence is a major constraint; attenuated rubella vaccine virus must be tested directly rather than inferred from wild-type RUBV; and temporal ordering is required. This addendum updates the cellular mechanism to the current two-bottleneck architecture and adds Paper VII without changing those disease-level safeguards.

14. CORRECTION TO THE ENDOLYSOSOMAL MECHANISM: BOTTLENECK #1

The September 20 model diagram and causal-test language place heavy emphasis on ARL8/HOPS → CI-M6PR → NPC2 → cholesterol egress. That host pathway remains mechanistically important, but the current Jacob model places Bottleneck #1 at LAL-centered lysosomal cholesteryl-ester/triglyceride throughput and capacity. The proposed slow handoff is after LAL hydrolysis and before NPC2 receives free cholesterol. NPC2 remains downstream and a competing discriminator.

The revised cellular question is therefore not simply whether RUBV reduces NPC2 availability. It is whether a RUBV-remodeled endolysosomal system develops an abnormal relationship between incoming CE/TG load, effective LAL processing capacity, free-cholesterol generation/residence, and post-LAL delivery to NPC2. LAL can be present, catalytically functional, or transcriptionally increased and still be rate-limiting if substrate load exceeds effective throughput or if product transfer becomes slow.

This distinction creates a hard falsifier. A meaningful LAL-throughput bottleneck requires a reproducible lysosomal CE/TG backlog or impaired intact-cell CE/TG clearance relative to incoming load. If no substrate queue or flux defect is present through robust mature infection, Bottleneck #1 is weakened regardless of LIPA transcript abundance.

15. UPDATED CAUSAL TEST #1 - RUBV MECHANISM

The RUBV mechanism test should retain P150-ARL8 perturbation and ARL8/HOPS, VPS39/VPS41, CI-M6PR, NPC2, pH, and membrane-chemistry measurements, but add direct substrate and flux resolution. Quantify lysosomal CE and TG load, particle-derived CE/TG delivery, direct LAL activity in intact-cell context, hydrolysis rate, free-cholesterol generation and residence, NPC2 receipt/transfer, NPC1-dependent export, ER arrival, SOAT re-esterification, and SREBP/sterol-feedback state.

A positive mechanism should identify where flux actually slows. If CE/TG backlog precedes downstream cholesterol starvation while NPC2 transfer remains competent, LAL-centered capacity is favored. If CE/TG hydrolysis is normal but free cholesterol accumulates before NPC2/NPC1 export, the downstream competitor gains support. If neither phenotype occurs, the proposed endolysosomal mechanism weakens.

16. PAPER VII ADDS BOTTLENECK #2: MAO

Paper VII introduces a second proposed bottleneck at the mitochondrial outer membrane: monoamine oxidase. RUBV directly engages and functionally perturbs mitochondria through capsid-p32 interaction, mitochondrial-surface capsid, inhibition of mitochondrial precursor-protein import, reduced intramitochondrial p32, and selective respiratory-chain remodeling. Independently, MAO function is experimentally coupled to mitochondrial membrane lipid context.

Host sterol biology supplies a plausible physical bridge: cholesterol-rich lysosomes form expanded STARD3-dependent contacts with mitochondria in NPC1-deficient or inhibited systems. This does not establish that Jacob's LAL/post-LAL lesion is equivalent to NPC1 deficiency, and it does not establish RUBV-induced MAO dysfunction. It establishes that lysosomal sterol state can alter lysosome-mitochondria contact biology in a defined host system.

The disease-level model may therefore test a second downstream cellular phenotype: altered MAO-A and/or MAO-B function associated with a defined mitochondrial outer-membrane state. MAO-A versus MAO-B remains an experimental discriminator. No recovered experiment directly demonstrates RUBV-induced MAO-A or MAO-B functional alteration through mitochondrial membrane lipid remodeling.

17. UPDATED INTEGRATED MODEL

WILD-TYPE RUBV or directly tested attenuated rubella vaccine strain -> RUBV replication-compartment maturation / P150-ARL8 foothold -> altered endolysosomal load, cargo, pH, or membrane state? -> LAL-centered CE/TG throughput/capacity and post-LAL/pre-NPC2 handoff? -> altered lysosomal cholesterol residence and downstream availability? -> altered sterol-responsive lysosome-mitochondria contact / mitochondrial membrane state? -> MAO-A and/or MAO-B functional change? -> neurodevelopmental consequence?

Every question mark is a required experiment. The current evidence does not establish this complete sequence in RUBV, in autism, or after MMR. Paper VI remains a causal hypothesis until the viral mechanism, human phenotype, and temporal sequence converge prospectively.

18. UPDATED HUMAN TEST

The human autism test remains centered on carefully phenotyped developmental regression versus non-regression and unaffected controls. Preserve the existing BMP/LBPA, sphingomyelin, ceramide, NPC2, lysosomal free-cholesterol, and LDL-derived ER-delivery measurements, but add CE/TG compartmental load and direct LAL-throughput markers capable of distinguishing substrate backlog from downstream transfer failure.

If technically and ethically feasible in an appropriate research system, mitochondrial/MAO measurements may be added as a separate Paper VII layer. A MAO phenotype must not be inferred from behavioral features, serum lipids, serotonin measures, or the endolysosomal phenotype alone.

19. UPDATED TEMPORAL TEST

The chronology requirement becomes more specific: proposed exposure -> RUBV biological state / replication-compartment maturation -> endolysosomal CE/TG and LAL/post-LAL phenotype -> any sterol-contact/mitochondrial membrane phenotype -> any MAO-A/MAO-B phenotype -> downstream neurodevelopmental change. A later node cannot be used to prove an earlier one, and co-occurrence cannot establish direction.

For any MMR extension, the attenuated rubella strain itself must reproduce the relevant upstream cellular state before downstream disease claims are considered. Existing population evidence still weighs strongly against MMR as a common cause of autism and remains a non-negotiable constraint on interpretation.

20. FOUR TESTS NOW SEPARATE THE FULL MODEL FROM DEMONSTRATION

CAUSAL TEST 1 - RUBV ENDOLYSOSOMAL MECHANISM: demonstrate and localize the LAL-centered CE/TG throughput/post-LAL defect during stage-resolved RUBV infection. CAUSAL TEST 2 - HUMAN AUTISM: demonstrate the predicted compartmental lipid/cholesterol phenotype in a prospectively defined developmental-regression subgroup. CAUSAL TEST 3 - TEMPORAL ORDERING / MMR: establish chronology and directly test the relevant attenuated rubella strain for any MMR extension. CAUSAL TEST 4 - MAO: demonstrate a RUBV-associated MAO-A and/or MAO-B functional phenotype, determine whether it tracks with mitochondrial membrane state, and test rescue or causal separation from Bottleneck #1.

Failure of Test 4 does not erase a validated endolysosomal mechanism; it rejects or narrows the MAO extension. Failure of Test 1 removes the central cellular bridge used by this Paper VI causal model. Failure of the human or temporal tests prevents assignment of the cellular mechanism to the proposed autism subtype even if the in-vitro RUBV mechanism is real.

21. UPDATED CONCLUSION

Paper VI now contains a stricter model than its September 20 version. The endolysosomal branch no longer assumes NPC2 is the lead bottleneck; it predicts a LAL-centered CE/TG throughput constraint with a proposed slow post-LAL/pre-NPC2 handoff and requires direct flux to distinguish that state from downstream NPC2/NPC1 alternatives. Paper VII adds a separately falsifiable mitochondrial/MAO branch whose RUBV-specific functional bridge remains unproven.

The disease-level conclusion remains bounded. Congenital RUBV supplies a human neurodevelopmental precedent elsewhere in the series, and human autism with developmental regression supplies a biologically relevant lipid phenotype. Those observations do not establish the complete causal sequence proposed here. RUBV causation of a non-congenital autism subtype through this pathway remains a testable causal hypothesis, not an established conclusion.

The model must now survive four separable challenges: direct RUBV flux mechanism, prospective human compartmental phenotype, exposure-to-phenotype chronology including direct vaccine-strain testing for any MMR extension, and the new MAO functional test. The value of the model is that each layer can fail independently and force the architecture to narrow.

ADDITIONAL REFERENCES FOR THIS ADDENDUM

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