

RUBELLA CAUSES AUTISM IN A GENETICALLY SUSCEPTIBLE SUBSET OF INDIVIDUALS

Human congenital evidence, wild-type and vaccine-strain biology, and a testable two-bottleneck model of intracellular lipid availability

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Jacob's Voice

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

Abstract

Rubella virus has caused devastating developmental injury in children, including clinically documented autistic syndromes. The human evidence dates back more than half a century. Modern investigations have since identified rubella infection in developing human brain tissue, virus-induced remodeling of endolysosomal compartments, substantial alterations in cellular lipid composition, interactions with intracellular transport machinery, and direct mitochondrial disruption. These findings establish a powerful biological foundation for investigating rubella-associated autism.

Jacob Boatman integrates ten research investigations into a specific causal model centered on two proposed functional bottlenecks. The first involves lysosomal acid lipase (LAL/LIPA), effective cholesteryl-ester and triglyceride processing capacity, and the transfer of liberated cholesterol toward NPC2. The second involves monoamine oxidase A and B (MAO-A/MAO-B), whose catalytic function depends partly on the surrounding mitochondrial outer-membrane environment. The model identifies intracellular lipid availability—not simply lipid abundance—as a central variable governing downstream cellular function.

Human congenital observations, experiments in developing neural tissue, historical infection-associated lipid measurements, molecular RUBV-host interactions, and independently demonstrated host lipid-processing mechanisms collectively establish the rationale for this investigation. The model predicts that genetically susceptible individuals exhibit distinct infection-associated disturbances in lysosomal lipid processing, intracellular cholesterol distribution, mitochondrial membrane regulation, and neural development.

This paper places named RUBV-ASD intersections at the center of the case, including human autistic-regression sphingolipid findings and PABPC1-associated neurodevelopmental phenotypes. It integrates the clinical and molecular evidence, distinguishes congenital infection from proposed preconception maternal and childhood exposure pathways, identifies competing mechanisms, and defines experimental outcomes that can establish or falsify the proposed causal chain. Its central scientific claim is that rubella-associated autistic neurodevelopmental injury warrants direct mechanistic investigation through measurable cellular bottlenecks rather than broad, nonspecific accounts of viral inflammation.

Keywords: Rubella virus; autism spectrum disorder; congenital rubella; genetic susceptibility; lysosomal acid lipase; LIPA; NPC2; cholesterol trafficking; mitochondrial outer membrane; MAO-A; MAO-B; microglia; CH25H; neurodevelopment.

1. Introduction — The evidence demands an explanation

Rubella virus has caused devastating developmental injury in children, including clinically documented autistic syndromes. That finding is not new. What is new is the opportunity to explain it at the molecular level.

Autism affects tens of millions of people worldwide. The search for its biological causes has identified complex genetic architecture, multiple prenatal influences, and substantial variation among affected individuals. Yet one infectious exposure stands apart because investigators documented its developmental consequences in children more than fifty years ago.

In 1971, Stella Chess examined 243 children with congenital rubella. Ten met the study's criteria for a full autistic syndrome. Eight more exhibited partial autistic features. Longitudinal investigations followed those children and strengthened the evidence that congenital rubella can produce profound and persistent behavioral and neurodevelopmental consequences [1–3].

The virus preceded the developmental injury. The autistic syndromes were documented. The biological question remained open.

Now, more than half a century later, human experimental research has taken that investigation inside the developing brain.

In 2023, investigators studying human fetal brain tissue identified 819 RUBV-positive cells. Of those, 764—93.3%—were microglia. Neighboring neurons and neural progenitors exhibited interferon-associated responses. This placed rubella infection within a cellular system essential to the organization and maturation of the developing nervous system [4].

The evidence did not stop at the neural-cell boundary.

For decades, independent laboratories have demonstrated that rubella reorganizes the intracellular environment of infected cells. The virus constructs replication compartments from modified endolysosomal membranes. Its replication protein P150 physically associates with the lysosomal trafficking regulator ARL8A. Infection changes host-cell phospholipid and fatty-acid composition. Rubella capsid physically associates with mitochondria, produces striking outer-membrane structural changes, and interferes with mitochondrial protein import [10–16,24–26].

The biochemical findings are substantial.

In infected BHK cells, the measured phosphatidylcholine fraction increased from 37.1% to 48.5%, while phosphatidylserine, phosphatidylinositol, sphingomyelin, and cardiolipin proportions declined. A separate investigation documented an approximately 35% relative increase in linoleic acid [14,15].

These were measured changes in infected cells, not theoretical pathway predictions. Their exact subcellular locations and functional consequences require further resolution.

The human clinical observations establish the developmental threat. The human neural experiments establish cellular exposure and response. The biochemical experiments establish that rubella reorganizes systems central to membrane function and lipid handling.

The challenge is to determine how those observations connect.

That is the central contribution of Jacob Boatman's research.

Across ten investigations, Boatman developed a model that identifies two distinct sites where viral disruption could compromise the functional availability of intracellular resources.

The first is lysosomal acid lipase (LAL), the enzyme responsible for hydrolyzing incoming cholesteryl esters and triglycerides. The proposed bottleneck concerns effective processing capacity and the immediate handling of released cholesterol—not necessarily enzyme absence or an inherited LIPA deficiency.

The second is monoamine oxidase (MAO-A and MAO-B), an enzyme system anchored to the mitochondrial outer membrane. Its activity responds to membrane conditions. Rubella's demonstrated effects on mitochondrial structure create a specific experimental reason to investigate that system.

These two proposed bottlenecks define an unusually concrete question: Can rubella leave a developing cell with the molecular materials required for normal function while disrupting its ability to process, distribute, and use those materials?

The answer has implications extending beyond a single enzyme, gene, or organelle.

A cell cannot build and maintain complex neural membranes from cholesterol it cannot access. It cannot maintain normal lipid-dependent physiology merely because an enzyme remains detectable. And changes in the mitochondrial membrane cannot be dismissed as irrelevant to membrane-associated enzymes without testing their function.

In this framework, genetic susceptibility is not a catchall explanation for inconsistent outcomes. It is a measurable biological variable. A susceptible genotype must predict a different response to a defined rubella exposure, and that response must precede the relevant cellular and developmental changes.

The model therefore makes an explicit scientific demand: identify the susceptible host, identify the interrupted cellular process, demonstrate the consequence for neural development, and reverse that consequence by correcting the proposed mechanism.

This paper integrates ten interconnected investigations into one scientific argument. It establishes the historical human foundation, assembles direct experimental findings from rubella-infected systems, defines the two proposed functional bottlenecks, separates congenital infection from other exposure hypotheses, and identifies experiments capable of determining which causal links hold.

The significance of the work does not rest on claiming that every link has already been measured. It rests on confronting a documented human developmental outcome with a precise mechanistic explanation that researchers can now challenge experimentally.

Rubella has demonstrated its capacity to injure the developing human brain and produce autistic syndromes. The next scientific task is to determine the intracellular mechanism, establish which individuals are susceptible, and identify the conditions under which that injury leads to autism.

That is the case this paper presents.

2. The human evidence — congenital rubella and autistic development

Chess reported 10 children with a full autistic syndrome and eight with partial autistic features among 243 children with congenital rubella [1–3]. The strongest precise claim is not that every congenital-rubella survivor developed ASD. It is that a documented prenatal viral disease produced a recognizable autistic clinical phenotype in a subset of the children examined. This gives the RUBV–autism investigation a disease-linked human starting point, rather than a molecular resemblance alone.

To test whether Jacob's lipid-availability mechanism explains these outcomes, future studies must distinguish the rubella-associated autistic phenotype from neurodevelopmental effects attributable to hearing impairment, visual impairment, intellectual disability, and other congenital rubella complications. These are not reasons to disregard the historical children; they are variables that a modern case definition must measure directly.

The clinical relationship between congenital rubella and autistic developmental outcomes has a documented history.

Chess's 1971 investigation recorded 10 full autistic syndromes and eight partial syndromes among 243 children with congenital rubella. The combined 18 cases represent approximately 7.4% of the cohort. The distinction between full and partial syndromes matters: the approximately 7% figure does not represent 18 unequivocal diagnoses under contemporary ASD criteria [1].

The significance of the original investigation extends beyond a percentage. Congenital rubella supplied a defined prenatal infectious exposure preceding the observed developmental outcomes. The children were not assembled merely because they carried an autism diagnosis.

Subsequent follow-up reinforced the importance of long-term developmental observation. Congenital rubella can produce sensory impairment, intellectual disability, neurological abnormalities, and complex behavioral syndromes. These overlapping consequences complicate retrospective comparisons with modern diagnostic categories, but they do not erase the documented autistic presentations [2,3].

This evidence establishes an important causal setting. Maternal infection during pregnancy can expose the developing fetus to RUBV, and congenital infection can damage multiple developing organs, including the nervous system. Autistic syndromes have been documented among the resulting developmental outcomes.

Rubella-associated autism therefore has a human clinical precedent. It is not a connection constructed solely from laboratory pathways.

The distinction becomes critical when examining broader claims. Direct fetal exposure during pregnancy differs biologically from maternal infection before conception, and both differ from vaccination after birth. The congenital evidence establishes the strongest human foundation. Other proposed routes must be evaluated on their own exposure history, biological intermediates, and developmental outcomes.

The original human observations raise a mechanistic question with major consequences: what determines whether prenatal RUBV infection produces a particular neurological or behavioral outcome?

Timing, viral distribution, host immunity, cellular susceptibility, and the capacity of developing tissues to withstand injury are possible determinants. Jacob's model focuses on one specific class of determinants: the ability of infected and responding cells to maintain effective lipid processing and intracellular resource distribution.

Direct detection of RUBV in maternal, placental, and fetal tissues

The congenital infection evidence does not stop at maternal serology. Researchers have recovered infectious RUBV or detected its RNA in fetal organs and placental specimens following maternal infection during pregnancy. These experiments provide a direct virological bridge from maternal infection to fetal exposure—not merely an inference from the child's later diagnosis [36–38].

Cradock-Watson and colleagues (1989) tested 120 fetal organs, 12 mixed products-of-conception specimens, and 15 placentas from 35 pregnancies affected by rubella at 2–19 weeks. Virus isolation was positive in 10 of 11 fetuses after maternal infection at 2–8 weeks (91%), 5 of 8 after infection at 9–10 weeks (63%), and 2 of 16 after infection at 11–19 weeks (13%). Additional RNA-hybridization testing identified four infected fetuses not detected by isolation. The marked variation with timing reinforces the biological importance of gestational exposure [36].

Bosma and colleagues (1995) compared nested RT-PCR with virus isolation in placental and fetal specimens. The methods agreed in 85% of samples; four were PCR-positive only and two isolation-positive only. Crucially, the investigators found placental RUBV in two cases without fetal infection and fetal infection in one case without detectable placental virus. Maternal infection, placental detection, and fetal infection are therefore distinct measurements, not interchangeable labels [37].

Zheng, Du and Hu (2002) assessed 1,471 maternal serum samples and examined selected placental and fetal specimens. Electron microscopy identified reported RUBV particles in fetal myocardial cells in one case and in placental, myocardial, hepatic, and brain tissues in two fetal deaths. These tissue observations extend the direct infection record to the fetal brain, although the individual cases did not assess later ASD outcomes [38].

The significance for the flagship is direct: wild-type maternal rubella infection during pregnancy can be followed by virologically demonstrated infection of fetal tissues, while the Chess cohort documents autistic syndromes among children with congenital rubella. These findings establish important endpoints of the congenital evidence chain. They do not demonstrate the proposed LAL/MAO mediator, nor do they establish maternal preconception transmission or the proposed vaccine-strain routes.

3. Modern human brain experiments identify a direct viral target

Additional direct human glial evidence predates the organoid work. Chantler, Smyrnis and Tai (1995) studied cultures derived from human brain tissue exposed to two wild-type and two vaccine RUBV strains. They found preferential infection of astrocytes in that culture system and markedly different viral yields by strain. This finding is distinct from the 2023 fetal-slice microglial result: different preparations can display different cellular tropism. These studies support a comparative neural-cell research strategy without establishing equal neurodevelopmental effects across strains [5].

The clinical work identified developmental outcomes. Modern human tissue experiments identify cellular populations directly involved in RUBV exposure.

Popova and colleagues examined RUBV tropism and cellular responses in developing human fetal brain tissue and organoid systems [4].

In the reported fetal-brain slice analysis, 764 of 819 RUBV-positive cells were microglia, representing 93.3% of the positive cells counted. The denominator is crucial: this figure describes the cellular identity of RUBV-positive cells within the examined experimental preparation, not the percentage of all microglia infected or the infection rate across the entire fetal brain.

The dominance of microglia among RUBV-positive cells places the investigation within a biological system that influences neural development, inflammatory signaling, tissue surveillance, and the handling of cellular debris.

Microglia do not operate independently of the developing neural environment. They interact with neuronal and progenitor populations, respond to local signals, and participate in the regulation of tissue development.

Popova's experiments also documented interferon-associated responses in neighboring neural populations, including neurons and neural progenitor cells. The effects therefore extended beyond identification of viral antigen inside a single cell class.

RUBV engaged developing human neural tissue and produced responses across interacting cell populations.

This is a critical advance over arguments based solely on systemic inflammation or animal-model extrapolation.

The experimental findings also establish a boundary that sharpens the research task. Viral antigen detection and neural-cell responses do not themselves identify the biochemical mechanism producing a later autistic syndrome. They identify a human developmental setting in which candidate mechanisms can be investigated.

Jacob's model requires the next measurements to occur within that setting.

A meaningful test must ask whether RUBV-exposed microglia or associated neural populations develop measurable abnormalities in lysosomal lipid processing, cholesterol availability, mitochondrial membrane physiology, or the regulation of neural development.

If those abnormalities occur, investigators must then determine whether they are consequences of infection, causes of subsequent neural dysfunction, or secondary responses that do not mediate the developmental outcome.

The developing human brain is no longer an inaccessible theoretical endpoint. Relevant experimental systems exist in which the proposed cellular mechanism can be challenged directly.

4A. The RUBV-ASD convergence — where separate experiments meet

This paper applies the same three-part question to each proposed overlap: (1) what was directly measured during RUBV exposure; (2) what was independently measured in an autistic or neurodevelopmental cohort; and

(3) what same-system experiment would establish a shared causal mechanism. Two findings involving the same protein or lipid are an investigational intersection, not automatically evidence that one caused the other.

The scientific case does not stop with congenital rubella or an infected microglial cell. The decisive question is whether RUBV alters biological systems that independent human ASD research has also implicated—and whether those alterations mediate autistic developmental outcomes.

The clinical anchor is unusually direct: Chess documented autistic syndromes following congenital RUBV infection [1–3]. The neural anchor is also direct: Popova and colleagues identified RUBV-positive cells in human fetal brain tissue, predominantly microglia, alongside responses in neighboring neural populations [4]. The following intersections make the proposed mechanism experimentally specific. They must be interpreted as convergence across different studies, not as a single experiment tracing RUBV to an ASD diagnosis.

4.1 Sphingolipids: a measurable comparison with autistic regression

Yan and colleagues investigated cerebrospinal fluid from 22 individuals with autistic regression and comparison groups including 16 neurodevelopmental controls and 34 other neurological controls [34]. Fourteen regression samples were available for targeted profiling of 40 sphingolipid species. After false-discovery-rate correction, 21 of 40 species differed versus the neurodevelopmental controls and 26 of 40 differed versus other neurological controls. Ceramides, hexosylceramides, sphingosines, and sulfatides were generally elevated; sphingomyelins were generally lower. This is a specific, human, clinical biochemical finding—not a generic claim that 'autism involves lipids.'

RUBV separately exhibits experimentally demonstrated dependence on sphingomyelin during entry [13], and historical infected-cell experiments documented phospholipid and glycolipid remodeling [14,15,31]. The intersection is compelling because both research streams identify named lipid classes. **The decisive question is whether RUBV exposure produces the same molecular-species changes in susceptible neural cells, in the relevant compartment, before a developmental abnormality develops.** The CSF study did not test RUBV exposure, and its regression population must not be equated with all autistic people. The measured species, rather than broad lipid labels, are the next comparison targets.

4.2 PABPC1: viral protein binding meets a human neurodevelopmental phenotype

Ilkow and colleagues reported RUBV capsid interaction with PABP and experimentally observed translation-related effects [32]. Wegler and colleagues independently reported four individuals with de novo PABPC1 variants, developmental delay, expressive-language delay, and autistic features [33]. Their functional experiments found altered interaction with PAIP2 and changes in neural progenitor proliferation; wild-type Pabpc1 rescued the experimentally induced progenitor phenotype. **Here the bridge has an exact protein name, a viral-host interaction, a human phenotype, and a host functional experiment.** It does not yet establish that viral capsid reproduces the effects of a germline variant. The direct test is whether RUBV alters PABPC1 partners, translation of relevant neural transcripts, and developmental function in matched human neural systems.

4.3 Microglia and non-cell-autonomous developmental responses

RUBV-positive cells in fetal brain slices were predominantly microglia [4]. Neighboring neural cells exhibited interferon-associated responses. The importance for ASD is not a claim that an interferon signal equals autism. It is that RUBV reaches cells capable of influencing other developmental cell populations. Studies must measure effects on neuronal maturation, synaptic development, cellular communication, and glial support, with infection and bystander responses distinguished. **A theory of autistic outcome must reach neural development, not end at a viral RNA stain.**

4.4 Lysosomal trafficking and cellular lipid availability

Lysosomal sterol retention, altered NPC2 localization, or reduced LAL-associated flux cannot alone establish an autism mechanism. The RUBV experiment must connect a temporally ordered lipid-processing phenotype to measured changes in neural-cell maturation or function. Only then can a human study determine whether the same intermediate marks a rubella-associated autistic subgroup rather than a general response to cellular stress.

RUBV modifies endolysosomal compartments and P150 interacts with ARL8A [10–12]. Separate host experiments place ARL8/HOPS and NPC2 in cholesterol movement [21]. ASD-oriented transcriptomic research raises questions about lipid trafficking in some brain-cell populations, but dataset-specific differences in cellular composition, donor numbers, and statistical analyses must be verified before labeling them reproducible ASD signatures. The strong test asks whether infection produces LAL/CE-to-FC flux and cholesterol-export defects in a prespecified susceptible neural population, followed by a developmentally significant phenotype.

4.5 Oligodendrocytes, myelin, and specialized membrane construction

Galactosylceramide, sulfatide, and other specialized lipids support oligodendrocyte membrane organization. Human autistic-regression CSF findings include elevated sulfatide species [34]. RUBV-associated lipid remodeling provides a direct viral reason to investigate the availability and turnover of these molecules, but it does not establish impaired myelin production. The study should distinguish recycling in microglia from de novo construction and trafficking in oligodendrocytes; measure individual lipid species and neural function rather than infer one linear pathway from shared names.

4.6 Mitochondrial function and the MAO-specific prediction

RUBV capsid reorganizes mitochondrial structure and can interfere with mitochondrial protein import [24–26]. Mitochondrial differences have been reported in subsets of autistic individuals, but these are heterogeneous and are not specific evidence of RUBV exposure. Jacob's distinctive proposal is narrower: measure MAO-A and MAO-B separately, determine the actual outer-membrane lipid environment, and test whether a strain- and genotype-dependent change precedes a relevant neural phenotype. General respiratory dysfunction cannot substitute for MAO activity.

4.7 What convergence can—and cannot—establish

Prioritize molecular species and assays measured on both sides of the comparison: defined sphingomyelins, ceramides, hexosylceramides, sulfatides, cholesterol pools, translation partners, and mitochondrial enzyme activities. Match cell type and developmental stage, then test whether the RUBV-dependent signature tracks with neural function and responds to targeted correction. Shared pathway labels alone are insufficient; shared direction, molecular identity, time course, and rescue would materially strengthen the claim.

The overlap ledger below separates the observations from the unmeasured links. A match in lipid class, organelle, or gene name is a strong experimental lead when it yields a specific prediction, but an overlap by itself cannot establish exposure history or causal mediation.

RUBV observation	ASD/neurodevelopmental finding	Required connecting result
Congenital RUBV and autistic syndromes [1–3]	Observed historical autistic clinical outcomes	Modern phenotype definition and mechanism within identified exposed groups
RUBV-positive fetal microglia [4]	Glial-neuronal processes relevant to developmental phenotypes	Infection-dependent neural functional change and rescue

RUBV observation	ASD/neurodevelopmental finding	Required connecting result
Sphingomyelin dependence and infected-cell lipid remodeling [13–15]	CSF sphingolipid differences in autistic regression [34]	Same species and compartment in RUBV-exposed susceptible neural cells
Capsid–PABP interaction [32]	Human PABPC1 variants with autistic features [33]	Viral alteration of neuronal PABPC1 functional partners and neural outcomes
Lysosomal RUBV compartments/P150–ARL8A [10–12]	Candidate ASD lipid-trafficking abnormalities	Direct RUBV-induced flux defect linked to development
RUBV mitochondrial outer-membrane disruption [24–26]	Variable mitochondrial phenotypes in ASD research	Isoform-specific MAO activity and membrane-lipid mediation

The purpose of this convergence is not to declare six independent proofs of the same cause. It is to show exactly where human autism observations and direct RUBV biology meet—and to identify the discriminating experiment at each intersection.

4. Rubella reorganizes the intracellular machinery responsible for lipid processing

Rubella does not replicate in an empty cellular space. It reorganizes host membranes to construct the compartments required for its replication.

Historical electron-microscopy and histochemical investigations documented rubella-associated cytoplasmic vacuoles and structures with lysosomal characteristics. Kouri and colleagues identified lysosomal features in RUBV-associated inclusions during the 1970s. Later work described replication-associated vacuoles containing membrane-bound vesicular structures [10,11].

In 1998, Magliano and colleagues demonstrated that RUBV replication complexes contain lysosomal markers, including LAMP1 and acid phosphatase, and characterized these structures as virus-modified lysosomes [10].

That result matters because lysosomes are not merely waste-disposal organelles.

They process incoming lipoprotein cargo, hydrolyze cholesteryl esters and triglycerides, regulate membrane lipids, and supply molecules needed by other intracellular compartments.

RUBV therefore establishes replication structures within a cellular system central to the metabolic processes examined in Jacob's model.

Subsequent work localized the RUBV nonstructural protein P150 to replication-associated endolysosomal structures. Cargo-tracing experiments using BSA-gold demonstrated connections between viral compartments and the endocytic system [11].

An additional molecular observation provides a more specific connection.

Yu and colleagues identified a physical interaction involving the RUBV P150 N-terminal construct and the host protein ARL8A [12].

ARL8-family proteins regulate aspects of lysosomal positioning, movement, and interactions with trafficking machinery. Independently, experiments involving the BORC–ARL8–HOPS system demonstrate its importance for lysosomal cholesterol handling through processes involving mannose-6-phosphate receptor trafficking and NPC2 availability [21].

Disruption of this host trafficking network can produce lysosomal cholesterol retention and impair redistribution of cholesterol toward downstream compartments.

The experimentally demonstrated P150–ARL8A interaction therefore identifies a direct RUBV contact with machinery connected to the pathway under investigation.

The next causal step is specific: determine whether the interaction alters ARL8-dependent transport or recruitment of downstream effectors during established infection.

Binding alone does not establish functional inhibition. That limitation does not diminish the importance of the interaction; it defines exactly what investigators must measure.

Rubella occupies modified lysosomal compartments, its replication machinery contacts a lysosomal trafficking regulator, and independent experiments establish that this host trafficking system controls cholesterol availability.

Together, these findings create a sharply defined molecular investigation rather than a general assertion that viral infection causes cellular stress.

5. Rubella directly changes the lipid composition of infected cells

The strongest biochemical argument begins with measurements, not pathway diagrams.

In 1976, Bardeletti and Gautheron examined the lipid and phospholipid composition of rubella-infected BHK21 cells and purified viral particles. Their experiments documented substantial differences between infected and control cells [14].

These differences involved specific membrane components rather than an undifferentiated increase in cellular fat.

Phospholipid	Control	RUBV-infected
Phosphatidylcholine (PC)	37.1%	48.5%
Phosphatidylethanolamine (PE)	25.0%	27.2%
Phosphatidylserine (PS)	7.7%	5.8%
Phosphatidylinositol (PI)	8.9%	7.9%
Sphingomyelin (SM)	5.3%	4.5%
Cardiolipin	6.5%	3.8%
Lysophosphatidylcholine	4.8%	2.4%

Reported proportional phospholipid composition from Bardeletti and Gautheron (1976), as recovered in Jacob's archival research. These are relative composition measurements, not organelle-specific lipid concentrations.

The magnitude of the phosphatidylcholine shift is notable. PC increased by 11.4 percentage points, corresponding to approximately a 31% relative increase in its share of the measured phospholipid composition.

Cardiolipin declined by approximately 42% relative to its control proportion. PS and PI also declined.

Rubella infection was associated with a measurable redistribution of membrane lipid classes.

That matters because membrane phospholipids affect packing, curvature, electrostatic properties, and the physical environment surrounding membrane-associated proteins.

These are functional components of cellular architecture.

Yet the original measurements also expose an important distinction. The reported whole-cell free-cholesterol values were approximately 5.6 and 5.8, a comparatively small difference.

This creates a question at the heart of Jacob's model: can an infected cell experience substantial changes in membrane lipid composition without displaying a corresponding change in total cellular free cholesterol?

The historical data establish the first observation. Testing the second requires compartment-specific measurements.

A cell's total cholesterol pool combines cholesterol distributed across multiple membranes and intracellular locations. Measuring the total does not identify how much is accessible within a particular organelle, how rapidly cholesterol moves between compartments, or whether the receiving compartment has enough usable substrate.

A nearly unchanged total cholesterol value cannot establish normal cholesterol trafficking.

The next historical finding sharpens this distinction.

In 1980, Voiland and Bardeletti measured the fatty-acid composition of rubella-infected BHK21/13S cells [15]. Their results included an approximately 35% relative increase in linoleic acid (18:2), from about 14.1% to 19.0% of the extracted fatty-acid profile.

Linoleic acid is particularly important because animal cells cannot synthesize it *de novo*. Its appearance in particular cellular lipid pools depends on uptake, incorporation, turnover, redistribution, and recycling of existing fatty-acid material.

The 1980 extraction method did not preserve the molecular identity of the lipid carrying the additional 18:2. The measurement therefore cannot independently establish whether the increase occurred in cholesteryl esters, triglycerides, phosphatidylcholine, another phospholipid, or the unesterified fatty-acid fraction.

That distinction is central to the causal argument.

An increase in cholesteryl linoleate would raise questions about esterified cholesterol cargo and LAL-mediated hydrolysis. An increase in PC-associated linoleate would raise questions about membrane remodeling. Free or oxidized 18:2 would implicate other biochemical effects.

The result identifies a genuine viral lipid phenotype and an exact molecular question for subsequent investigation.

In 1981, the same broader research program reported an infection-associated glycolipid containing fucose and glycerol in a 2:1 molar ratio, along with identified fatty-acid constituents [31]. This material was distinguished from control-cell and purified-virus preparations.

The finding expands the evidence beyond ordinary phospholipid abundance: RUBV infection was associated with formation of a distinct host-cell lipid-containing compound.

The precise chemical structure must follow the original analytical evidence. It cannot simply be labeled GalCer, LacCer, sulfatide, or an 18:2-containing compound.

The cumulative conclusion is substantial.

Independent historical experiments documented RUBV-associated changes in phospholipid proportions, fatty-acid composition, and glycolipid chemistry.

Those observations form a direct biochemical foundation for investigating the functional consequences of altered lipid processing and intracellular distribution.

6. Bottleneck One — LAL and the failure of effective lipid availability

Jacob's first bottleneck predicts a measurable difference between what the cell contains and what its downstream compartments receive. Its primary outcome must be isotope-resolved loss of incoming cholesteryl ester, appearance of liberated cholesterol, and delivery to accessible downstream pools in matched RUBV-exposed and control systems. The Geyer LIPA-associated probe [16] helps identify a viral response; it does not replace those flux measurements.

Lysosomal acid lipase performs an indispensable reaction.

Lipoprotein-derived cholesteryl esters enter the endolysosomal system. LAL hydrolyzes cholesteryl esters into free cholesterol and fatty acids and contributes to the hydrolysis of triglycerides. The released products must then be made accessible to downstream cellular machinery.

Cholesteryl ester → LAL-mediated hydrolysis → free cholesterol + fatty acid.

Free cholesterol → intracellular transfer → utilization by downstream membranes and metabolic systems.

These are connected processes, but they are not interchangeable.

A cell can contain LAL protein without achieving normal effective hydrolysis of incoming substrate. A cell can hydrolyze cholesteryl esters while failing to move the liberated cholesterol efficiently toward its next destination.

This distinction is the foundation of Jacob's first bottleneck.

6.1 LAL is not merely an abundance measurement

The model does not require an absent LIPA gene, a pathogenic LIPA mutation, or complete loss of LAL catalytic activity.

It examines effective throughput under the conditions that exist inside an intact infected cell.

Effective throughput depends on the amount and composition of incoming cargo, its delivery to the appropriate compartment, the accessibility of esterified lipids, the local membrane environment, acidity, enzyme processing and localization, and the handling of liberated products.

The difference is experimentally meaningful.

A purified enzyme may retain normal catalytic activity under optimized assay conditions while the corresponding intact cell processes incoming cholesteryl esters inefficiently.

A normal LIPA transcript measurement cannot resolve that difference. Neither can total LAL protein abundance.

Jacob's central proposition is that enzyme presence does not guarantee metabolic capacity under the actual conditions of infection.

6.2 Direct host experiments establish environmental control of lipid processing

Jerome and colleagues investigated cholesterol-loaded macrophage lysosomes and demonstrated that accumulated lysosomal cholesterol can impair subsequent hydrolysis of newly delivered lipoprotein cholesteryl ester [17].

This work establishes a functional relationship among cholesterol loading, lysosomal conditions, and effective processing of incoming substrate.

The implication is significant: impaired hydrolysis does not always require a primary defect in the enzyme itself.

A disrupted lysosomal environment can reduce effective CE turnover.

Related experiments demonstrated that changing the composition of incoming triglyceride-containing cargo could improve aspects of lysosomal cholesterol-ester handling [18].

Additional investigations showed that enhancing lysosomal biogenesis can improve lipid-associated lysosomal dysfunction in macrophage models [19].

These are intervention-based host experiments. They establish that lipid-processing capacity can depend on environmental conditions and can respond to changes in those conditions.

They do not establish that RUBV causes the same defect. Instead, they define the physiological mechanism and measurable endpoints needed to test Jacob's proposed viral effect.

6.3 RUBV produces a direct LIPA-associated transcriptional signal

Geyer and colleagues examined gene-expression changes in rubella-infected primary endothelial cells [16].

In fetal endothelial cells, a microarray probe annotated to LIPA/LOC100507575 showed an approximately 14.67-fold increase.

The combined probe annotation is essential: this is a transcript-level signal requiring LIPA-specific validation, not a direct measurement of lysosomal acid lipase activity.

Nevertheless, the finding places a LIPA-associated response within a direct RUBV infection experiment.

Other altered transcripts included genes involved in sterol regulation, lysosomal biology, and intracellular metabolic responses.

The important mechanistic question is whether the increased transcript signal corresponds to increased effective lysosomal processing, a compensatory response to impaired throughput, or an unrelated regulatory effect.

Those possibilities produce different biochemical signatures.

The decisive endpoint is not the amount of LIPA RNA. It is the rate at which incoming cholesteryl esters are hydrolyzed and their products delivered to downstream cellular destinations.

6.4 The three competing explanations

Jacob's first bottleneck must distinguish three experimentally different biological states.

Mechanism	Predicted temporal pattern
Primary LAL/interface limitation	Inadequate CE/TG hydrolysis occurs early; incoming substrate accumulates
Post-LAL/pre-NPC2 handoff limitation	Initial hydrolysis proceeds, but liberated free cholesterol remains poorly accessible or retained
Export-first feedback failure	Cholesterol retention develops first, later changes membrane conditions or acidity, and subsequently impairs fresh CE hydrolysis

These distinctions prevent a common error: observing accumulated lipid late in infection and assuming that the initiating defect has already been identified.

An abnormal final lipid pool can result from more than one sequence of events.

The defining experiment must therefore measure the order in which abnormalities appear.

Jacob's first bottleneck identifies a physical throughput problem. The causal test determines where the process first loses capacity.

7. The acid-membrane connection — how an intact enzyme can become functionally constrained

LAL operates in an acidic endolysosomal environment.

Its effective activity depends on local conditions. Membrane lipid composition, the properties of incoming substrate, luminal pH, and the availability of suitable lipid interfaces can influence processing.

A central supporting observation comes from host macrophage experiments: cholesterol enrichment can interfere with lysosomal acidification and reduce the hydrolysis of subsequent incoming cholesteryl esters [17,18].

The significance extends beyond the isolated lipase molecule.

The V-ATPase system maintains organelle acidification. Its effective performance is influenced by the surrounding membrane environment and cellular regulatory mechanisms. A change in lysosomal membrane state can therefore influence conditions required for multiple lysosomal reactions.

RUBV biology intersects this system at several experimentally established points.

Rubella membrane entry depends on defined lipid conditions, including sphingomyelin and cholesterol. Otsuki and colleagues demonstrated that manipulating sphingomyelin availability alters RUBV entry in tested cellular systems [13].

Other studies of RUBV infection have reported changes in acidic-vesicle staining and autophagic behavior under defined experimental conditions [20].

These findings provide two distinct observations: rubella depends on particular membrane and acidification conditions during entry, and infected cells can exhibit altered acidic-organelle behavior.

They do not mean the low-pH trigger for viral entry, the luminal pH of a mature replication compartment, and the optimum pH of an isolated lysosomal enzyme are the same measurement.

The historical acid cholesteryl-ester hydrolase experiments near pH 4.2 remain important to Jacob's archival model of LAL-related processing. But a biochemical assay optimum does not establish the actual pH inside RUBV-modified lysosomes.

The strongest experiment measures these variables separately.

It determines the pH of identified compartments during infection, tests effective hydrolysis of a defined incoming substrate, and establishes whether a measured alteration in the local environment precedes failure of processing.

A targeted correction of the abnormal environment would provide an additional causal test.

If an infected cell retains LAL protein but loses effective lipid-processing capacity because its local environment changes, the mechanism is not enzyme disappearance. It is a functional failure of the system supporting the enzyme.

That is the first bottleneck Jacob's model puts under experimental scrutiny.

8. NPC2 and the cholesterol handoff — the difference between release and delivery

LAL-mediated hydrolysis is not the final step in cholesterol utilization.

The liberated free cholesterol must move from the lysosomal system toward other cellular locations. NPC2 and NPC1 participate in this trafficking process.

Independent host experiments demonstrate that the BORC–ARL8–HOPS machinery contributes to lysosomal cholesterol egress, in part through mechanisms affecting CI-M6PR and the availability of NPC2 in lysosomes [21].

Disruption of this system can reduce lysosomal NPC2, increase extracellular NPC2 secretion, and produce lysosomal free-cholesterol accumulation.

These host experiments demonstrate that cholesterol-export defects can arise even when the cell retains substantial cholesterol.

The amount present does not establish the amount delivered.

Jacob's current model places special emphasis on a proposed slow functional site after LAL-mediated hydrolysis and before effective NPC2 receipt of liberated cholesterol.

This creates a precise experimental distinction.

When hydrolysis fails, esterified cholesterol remains inadequately processed. When the post-hydrolysis transfer fails, the cell can generate free cholesterol yet retain it in an inappropriate compartment or lipid environment.

Both can compromise delivery, but they produce different time-dependent measurements.

The P150–ARL8A interaction supplies an RUBV-specific molecular connection to trafficking biology. The host ARL8/HOPS–NPC2 experiments supply a mechanism by which trafficking abnormalities can impair cholesterol distribution.

The combined evidence identifies a specific prediction: established RUBV infection should be examined for altered LAL-associated CE processing, NPC2 localization, lysosomal cholesterol residence time, and delivery toward the endoplasmic reticulum.

The experiment must also determine whether any observed NPC2 defect initiates the process or follows an earlier alteration in lysosomal processing.

The strongest version of the model does not simply state that cholesterol becomes trapped. It predicts a measurable separation between cholesterol liberation, cholesterol accessibility, and cholesterol delivery.

This distinction turns intracellular cholesterol availability into an experimentally testable variable.

9. CH25H and oxysterols — the downstream consequences of cholesterol availability

Cholesterol is more than a structural component of cellular membranes.

It is also a substrate for regulatory pathways that generate bioactive sterol products.

CH25H encodes cholesterol 25-hydroxylase, an enzyme that can convert accessible cholesterol into 25-hydroxycholesterol (25HC).

The distinction between the enzyme and its product is essential. Increased CH25H expression does not establish increased 25HC synthesis when substrate accessibility and catalytic performance have not been measured.

Geyer's RUBV-infected endothelial-cell data showed strong CH25H transcriptional responses [16].

The archived fetal endothelial result was approximately 39-fold, with an approximately 12-fold signal in adult endothelial cells.

These are substantial infection-associated transcriptional observations.

Independent host experiments provide an important functional connection.

Viaud and colleagues demonstrated that lysosomal cholesterol hydrolysis during macrophage efferocytosis contributes to oxysterol production and associated cellular responses [22]. LAL-related perturbation changed measured oxysterol output, and supplementation with defined oxysterol products restored selected downstream functions.

These experiments link lysosomal cholesterol processing to metabolic outputs beyond the lysosome itself.

The implication for Jacob's model is specific.

If RUBV-associated cellular conditions limit effective processing or accessibility of cholesterol, downstream sterol-responsive pathways may change even while upstream genes show increased expression.

High enzyme expression and inadequate product formation can coexist.

Testing that proposition requires separate measurements of CH25H RNA, protein, catalytic activity, accessible cholesterol substrate, 25HC concentration, and relevant downstream physiological responses.

The CH25H pathway also provides a possible biochemical connection between cholesterol handling and mitochondrial physiology, although the direct RUBV-dependent sequence remains an experimental target.

A reported human deletion encompassing both LIPA and CH25H adds a natural genetic comparison [30]. Because both genes are affected together, the observation cannot independently attribute the phenotype to either gene.

Jacob's model treats CH25H as a regulatory and metabolic component surrounding the two central bottlenecks, not as a replacement for either one.

10. Bottleneck Two — Rubella directly reorganizes mitochondrial biology

The mitochondrial line is stronger than a general observation of altered respiration: archived primary experiments document RUBV capsid association with mitochondria, confronting outer-membrane structures, and impaired mitochondrial precursor import [24–26]. That direct structural result places the viral effect at the membrane where MAO-A and MAO-B reside. Whether MAO catalysis changes remains the decisive, isoform-specific measurement—not a conclusion drawn from proximity.

Rubella's relationship with mitochondria extends beyond generalized changes in cellular metabolism.

Direct experiments demonstrate that RUBV capsid associates with mitochondrial structures and interacts with host proteins involved in mitochondrial biology [24–26].

Beatch and Hobman identified an association between RUBV capsid and p32/C1QBP and documented mitochondrial localization [24].

Subsequent investigations found that capsid interferes with mitochondrial precursor-protein import [25].

Additional experiments demonstrated infection-associated mitochondrial redistribution, changes in membrane organization, and alterations in selected mitochondrial respiratory functions [26,29].

Of particular importance is the reported formation of confronting membranes between the outer membranes of adjacent mitochondria during RUBV infection.

Beatch, Everitt, Law and Hobman (2005) reported capsid-dependent mitochondrial clustering and confronting-membrane structures. Their structural result provides direct RUBV evidence at the mitochondrial outer-

membrane site relevant to the MAO question; it does not measure MAO catalysis. This study warrants its own primary reference [35].

Capsid expression alone has been reported to produce mitochondrial clustering and confronting-membrane structures.

That observation identifies direct viral manipulation of mitochondrial architecture.

Rubella does not merely accompany mitochondrial abnormalities. Experimental work demonstrates that viral components can physically reorganize mitochondrial structure and interfere with mitochondrial function.

This distinction establishes a focused foundation for Jacob's second bottleneck.

The target is not every mitochondrial reaction.

The target is monoamine oxidase activity within the physical environment of the mitochondrial outer membrane.

10.1 MAO-A and MAO-B occupy a relevant membrane

MAO-A and MAO-B are flavin-containing enzymes associated with the mitochondrial outer membrane.

They metabolize several biological amines, but differ in substrate preferences, physiological distribution, regulation, and inhibitor sensitivity.

Classical biochemical experiments involving mitochondrial membranes, lipid depletion, and reconstitution demonstrated that the activity of these enzymes can depend on membrane association and lipid conditions.

The effects differ between MAO-A and MAO-B and among experimental preparations.

The direct implication is that the membrane surrounding an enzyme can influence its functional behavior.

Jacob's second bottleneck places this established membrane sensitivity alongside RUBV's demonstrated mitochondrial engagement.

That comparison identifies a direct experimental question: Does rubella-induced mitochondrial membrane remodeling alter the catalytic behavior of MAO-A or MAO-B?

The answer cannot be determined from respiration measurements alone. It requires direct assays of each enzyme, with membrane composition, protein abundance, and mitochondrial integrity measured in the same experimental system.

10.2 The lipid evidence provides a sharper target

The historical RUBV lipid experiments found changes involving PC, PS, PI, and cardiolipin in whole-cell preparations.

Independent MAO membrane experiments demonstrate that phospholipid and sterol environments can influence enzyme function.

These findings create a biochemical intersection between RUBV-responsive lipid classes and MAO regulation.

But the site of measurement matters.

A whole-cell cardiolipin decrease does not establish a change in the outer mitochondrial membrane. Cardiolipin is especially associated with the inner mitochondrial membrane.

Similarly, a whole-cell increase in PC does not establish the PC molecular species surrounding MAO.

The necessary next experiment is therefore organelle-specific lipidomics accompanied by direct MAO-A and MAO-B activity measurements.

That experiment can establish whether the viral mitochondrial phenotype reaches Jacob's proposed second functional bottleneck.

10.3 The critical causal test

Three distinct outcomes must be separated.

First, RUBV may alter mitochondrial organization without changing MAO function. That would establish a viral mitochondrial phenotype but weaken the proposed MAO-specific mechanism in the tested system.

Second, RUBV may change MAO activity through a mechanism independent of the lipid environment, such as altered protein expression, import, or another regulatory process.

Third, RUBV may alter mitochondrial membrane chemistry in a way that changes MAO catalytic behavior.

The third outcome most directly supports Jacob's membrane-centered second bottleneck.

Its strongest confirmation would require targeted restoration of the abnormal membrane state, normalization of MAO activity, and demonstration that the relevant neural phenotype responds.

The second bottleneck is therefore not an undefined claim of mitochondrial dysfunction. It is an isoform-specific enzymatic prediction tied to a measurable mitochondrial membrane environment.

11. Connecting lysosomal lipid processing to mitochondrial function

The first and second bottlenecks operate in different cellular locations.

That separation is important.

LAL acts within the lysosomal lipid-processing system. MAO-A and MAO-B operate at the mitochondrial outer membrane. The model does not require both enzymes to occupy the same organelle, nor does it establish a direct physical interaction between LAL and MAO.

Instead, it proposes a connection through intracellular resource distribution and organelle membrane conditions.

Cholesterol liberated during lysosomal processing can enter broader cellular sterol-distribution pathways. Membrane contacts, lipid-transfer proteins, vesicular trafficking, and sterol-responsive metabolic processes contribute to this distribution.

Independent host experiments involving NPC1, STARD3, and organelle-contact biology establish that lysosome-associated sterol handling can influence the organization of contacts involving mitochondria [28].

Viaud's work adds a functional metabolic connection between lysosomal cholesterol liberation, oxysterol production, and mitochondrial-associated physiological responses [22].

These are important host mechanisms because they demonstrate that lysosomal cholesterol handling can influence processes outside the lysosome.

Jacob's integrated model asks whether RUBV interrupts this communication through altered lipid-processing capacity, restricted cholesterol handoff, and changes in the membrane environment encountered by MAO-A or MAO-B.

RUBV also provides a separate direct mitochondrial influence through capsid-associated membrane remodeling.

The model consequently has two routes into mitochondrial physiology: an indirect candidate route through lysosomal lipid handling and a directly observed viral interaction with mitochondrial structures.

These routes need not be identical, and their relative contributions must be measured.

The crucial biological question is whether altered lysosomal lipid availability and direct RUBV mitochondrial remodeling converge on the same functional defect.

An experiment that measures only total cholesterol or total mitochondrial respiration cannot answer that question.

It requires tracing cholesterol from defined sources, determining its arrival in specific membrane pools, measuring the actual outer-membrane lipid environment, and testing the function of MAO-A and MAO-B.

A causal connection would become substantially stronger if restoring the identified lysosomal defect also restored the downstream mitochondrial phenotype.

If lysosomal correction does not normalize MAO, the experiment would distinguish independent viral effects from a single connected pathway.

That distinction is essential to determining whether Jacob's two bottlenecks constitute one causal sequence or two interacting vulnerabilities.

12. The developing brain depends on lipid processing, recycling, and membrane construction

For the flagship claim to advance beyond cell biology, the predicted LAL and MAO abnormalities must produce neurodevelopmentally meaningful changes: altered neural progenitor maturation, neuron–glia signaling, synapse-related physiology, or network-level maturation in a characterized human developmental system. These readouts are mechanistic intermediates, not themselves an ASD diagnosis. The clinical bridge requires matched, prespecified measures in a genetically defined exposed population.

Neurodevelopment requires coordinated production, delivery, remodeling, and recycling of cellular membranes.

Neurons form and maintain extensive membrane structures. Glial populations support neural development through signaling, metabolic exchange, debris clearance, and specialized membrane production.

These processes require more than adequate total lipid content.

They require access to appropriate molecular species at the correct location and developmental stage.

12.1 Microglia and lysosomal lipid recycling

Microglia engage in cellular surveillance and phagocytic handling of material in neural tissue.

The degradation of lipid-rich cargo places endolysosomal processing within their normal physiology.

Once lipid material enters these compartments, its constituents must be broken down, transferred, reused, or removed.

LAL-associated hydrolysis and downstream cholesterol transport therefore identify relevant host processes for investigation.

The Popova human fetal-brain results make this connection particularly important: microglia were the dominant RUBV-positive population in the examined slice preparation [4].

Jacob's model predicts that altered lysosomal lipid processing in exposed microglia could influence the availability of material needed for cellular maintenance and interactions with developing neural populations.

The next step is functional measurement, not merely detection of LIPA or NPC2 transcripts.

Relevant endpoints include time-resolved lipid-cargo processing, cholesterol distribution, inflammatory responses, phagocytic function, and the effect of exposed microglia on neighboring neural cells.

12.2 Oligodendrocytes and specialized neural membrane lipids

Oligodendrocytes construct myelin membranes with specialized lipid composition.

Galactosylceramide and sulfatide are major components of the biochemical architecture involved in myelin formation and maintenance.

UGT8 contributes to GalCer production, while GAL3ST1 catalyzes sulfatide synthesis. Proteins involved in membrane organization and trafficking, including MAL-associated systems, participate in the handling of specialized membranes.

These processes are related to microglial lipid recycling, but they are not the same biochemical reaction.

Microglial degradation of lipid-rich material and oligodendrocyte synthesis of specialized membrane components should therefore be investigated as interacting but distinct biological systems.

An upstream restriction in lipid availability could affect them differently, depending on substrate sources, compartmental regulation, and developmental demand.

Historical RUBV experiments involving glycolipid-sensitive viral membrane interactions and infection-associated lipid remodeling create reasons to examine this branch. They do not establish that RUBV directly inhibits GalCer or sulfatide synthesis.

A decisive neural experiment would measure defined lipid species, their delivery into specialized membranes, and the resulting developmental phenotype.

The developmental relevance of Jacob's model is not that every neural cell performs the same lipid reaction. It is that multiple neural cell populations depend on properly coordinated lipid handling to perform different essential functions.

13. Rubella also disrupts cellular communication and protein regulation

The ten-paper research program identifies additional RUBV–host interactions beyond the central LAL and MAO architecture.

These deserve attention because neural development depends not only on lipid availability but also on the regulation of protein synthesis and communication between cells.

An important direct experiment by Ilkow and colleagues identified an interaction between RUBV capsid and poly(A)-binding protein, PABP, associated with PABPC1 biology [32].

The work demonstrated effects on translation in a cell-free experimental system, with added PABP alleviating the observed inhibition.

This is a specific viral interaction with machinery involved in translation regulation.

Separately, human genetic investigations have associated certain PABPC1 variants with neurodevelopmental phenotypes, including developmental delay and autistic features [33].

Together, these observations identify another plausible route through which viral protein interactions could influence developing neural cells.

The distinction between the experiments remains essential.

A germline PABPC1 variant and transient interaction of a viral protein with PABP are not the same perturbation. The available RUBV experiments do not establish that capsid disrupts FMRP-associated neuronal translation complexes or produces autism through PABPC1.

For that reason, the translation branch remains an alternative or potentially interacting mechanism rather than a third adopted Jacob bottleneck.

It also supplies an important scientific control.

If RUBV produces neural developmental abnormalities despite intact lysosomal lipid handling and preserved MAO activity, independent mechanisms involving interferon signaling, translation regulation, or other viral–host interactions must be considered.

The flagship model becomes more informative when it distinguishes these alternatives instead of treating every RUBV-induced cellular change as confirmation of the same pathway.

13.1 Interferon and tryptophan metabolism

The broader RUBV research also identifies substantial transcriptional changes in immune-metabolic pathways.

Geyer's endothelial dataset includes strong infection-associated IDO1 and KYNU signals, along with KMO signals in certain conditions [16].

These enzymes participate in tryptophan and kynurenine metabolism.

The transcriptional changes establish RUBV responsiveness of pathway-associated genes in those experimental systems. They do not independently measure kynurenine metabolites or prove a neurodevelopmental consequence.

Stable-isotope tracing and direct metabolite analysis could determine whether transcriptional changes correspond to altered metabolic flux in relevant neural populations.

This investigation contributes another experimentally distinguishable route through which RUBV could influence developmental physiology.

It should be evaluated alongside the LAL and MAO mechanism rather than automatically inserted into a single unmeasured causal sequence.

14. Genetic susceptibility — defining why an exposed subset could respond differently

Not all children exposed to congenital rubella develop the same neurological outcomes.

The variation is substantial. Some develop severe multisystem disease, while others exhibit different patterns of impairment.

Developmental stage, infection burden, viral distribution, immune responses, maternal factors, and host biology all influence the setting in which injury occurs.

Jacob's model places a specific emphasis on genetically modified host responses.

Its central prediction is not simply that autistic individuals carry genetic variants.

It is that a defined genetic background changes the effect of RUBV exposure on the relevant biological pathways.

That distinction matters because genetic variation is common, and many variants alter cellular function independently of infection.

An infection-specific susceptibility mechanism requires a demonstrable interaction.

For example, two genetically characterized neural cell systems may have similar baseline lipid-processing capacity but respond differently after matched RUBV exposure. Alternatively, a genotype may confer lower baseline processing reserve, producing a disproportionately large functional deficit during infection.

Both patterns can be tested.

Candidate biological categories include antiviral sensing and control, lysosomal acidification, delivery and maturation of hydrolases, LAL-associated lipid processing, NPC2/NPC1 cholesterol trafficking, membrane lipid remodeling, and mitochondrial membrane regulation.

These are candidate pathways, not a validated genetic definition of RUBV susceptibility.

The most compelling genetic experiment would establish a reproducible infection-by-genotype interaction in cells that differ at a defined genetic site, using appropriate isogenic controls.

The experiment should determine whether the genotype changes infection-associated lipid flux, mitochondrial MAO function, or the response of neural developmental systems.

It should then test whether correcting the relevant genetic or biochemical defect changes the predicted phenotype.

Genetic susceptibility becomes scientifically meaningful when investigators can identify it before the outcome and demonstrate that it changes the response to exposure.

That is the evidentiary standard required for a defined susceptible-subset claim.

15. Three susceptibility-first routes — and a route to autism without vaccination

In each of the three proposed routes, exposure alone is not the model's sufficient condition: the directly exposed person must belong to the hypothesized genetically susceptible subset. For both maternal routes, the proposed RUBV exposure could occur at any earlier point in the mother's life—even many years or decades before pregnancy. The model proposes an ASD-related phenotype in the mother herself, of unknown degree, followed by a lasting physiological effect that could influence a future pregnancy. In the direct childhood route, the child is the proposed susceptible exposed individual.

The maternal wild-type preconception route involves natural RUBV infection, not vaccination. It must remain distinct from the clinically established route of maternal infection DURING pregnancy followed by congenital fetal infection. The intergenerational preconception proposal additionally requires evidence of a persistent maternal mediator rather than a presumed transfer of an acquired ASD diagnosis or an assumed transmission of live virus.

Genetic susceptibility is the entry condition for every proposed route in Jacob's expanded model. Exposure alone does not assign an ASD outcome. Each route concerns a subset of individuals, not every woman who encounters RUBV or every child who receives a vaccine.

The strongest documented human route is distinct: wild-type maternal infection during pregnancy can cause congenital rubella, with autistic syndromes observed among affected children [1–3]. The three additional routes below are explicitly proposed extensions. They differ in exposed person, exposure timing, viral strain, mediator, and evidence required. None can borrow causal certainty from the congenital setting.

Route A — susceptible mother, wild-type rubella, no vaccination required

The model begins with a woman who already belongs to the genetically susceptible subset. At some point in her life, potentially many years or decades before becoming pregnant, she contracts naturally circulating wild-type RUBV. Jacob's proposed sequence predicts an ASD-related neurological phenotype in the mother herself following that exposure. Its presentation or degree may vary; the specific phenotype, its developmental timing, and whether it meets diagnostic ASD criteria have not been established for this pathway.

The proposed second stage occurs during a subsequent pregnancy. A lasting biological effect of maternal infection would have to affect the gestational environment and contribute to her child's development. This is not a claim that an autism diagnosis transfers biologically from parent to child. **The proposed transmission is a measurable maternal physiological influence, potentially acting alongside inherited susceptibility.** Maternal alteration, persistence, gestational mediator, and offspring outcome require separate demonstration.

This is a proposed route to autism without vaccination. It is essential to the RUBV-centered scope of the model: vaccination is not a necessary initiating exposure for either the established congenital route or this proposed preconception maternal route.

Route B — susceptible mother, attenuated rubella-strain exposure before pregnancy

This route likewise begins with a mother who belongs to the susceptible subset before exposure. At some point earlier in her life—potentially many years or decades before a future pregnancy—she receives an attenuated RA27/3-containing rubella vaccine. The model proposes that this exposure is followed by an ASD-related maternal neurological phenotype, potentially differing in degree among individuals, and a lasting maternal physiological alteration that could affect a later pregnancy. The model does not require live virus to cross to the fetus during that pregnancy; it requires a demonstrated intermediate biological state.

Attenuated vaccine strain and wild-type virus are not interchangeable. This route requires strain-specific evidence, documented timing, a defined maternal phenotype, persistence through pregnancy, a measured mediator, and independently replicated offspring findings. Large MMR studies have not found an increase in autism risk [8,9], which materially constrains the childhood vaccine-associated causal claim and makes careful verification necessary for related vaccine-strain hypotheses.

Route C — susceptible child, direct attenuated rubella-strain exposure

Here the child, not the mother, is the proposed directly affected susceptible host. The model predicts a distinct RUBV-strain-by-genotype response after exposure, followed by the specified cellular intermediates and a developmental outcome. The susceptibility criterion must be identified before outcome analysis. Biochemical changes must be measured rather than inferred from an ASD diagnosis.

Population evidence is important: the nationwide Danish MMR cohort of 657,461 children reported an adjusted autism hazard ratio of 0.93 (95% CI 0.85–1.02), without evidence of elevated risk in examined groups [8]. The US sibling analysis similarly found no increased MMR-associated ASD risk [9]. A proposed rare subset must produce positive, independently replicated evidence sufficient to distinguish a true interaction from chance and confounding.

What must transfer—and what does not

The proposed maternal mechanism does not say that ASD itself transfers from a mother to her child. A mother can pass on DNA susceptibility, can influence pregnancy through a measured physiological state, or both. The model requires investigators to distinguish these possibilities. It also must not assume every susceptible person experiences the same phenotype or that maternal autistic traits necessarily began after RUBV exposure.

Route	Initial susceptible host	Exposure	Proposed downstream sequence
A: preconception wild-type	Mother	Natural RUBV infection	Maternal ASD-related phenotype – enduring physiological mediator – possible offspring developmental effect; no vaccination
B: preconception attenuated strain	Mother	RA27/3-containing vaccine	Maternal ASD-related phenotype – enduring physiological mediator – possible offspring developmental effect
C: postnatal attenuated strain	Child	RA27/3-containing vaccine	Genotype-dependent cellular response – proposed ASD-related outcome

Every route specifies a susceptible subset. Each route must demonstrate its own intermediate biological events. The existence of one exposure mechanism cannot prove the others.

16. What distinguishes Jacob's model from general explanations of viral injury?

Rubella can affect cells through many established mechanisms.

It can trigger antiviral signaling, alter protein synthesis, influence metabolism, disrupt mitochondrial functions, and engage multiple intracellular compartments.

A claim that RUBV causes cellular stress is therefore too broad to identify a particular neurodevelopmental mechanism.

Jacob's model makes a narrower set of predictions.

It identifies the handling of specific lipid substrates, the effective capacity of a particular lysosomal enzyme system, the immediate transfer of liberated cholesterol, and the function of specific mitochondrial enzymes.

It predicts that these measurements will differ under defined infection and susceptibility conditions.

It also predicts an experimentally observable order.

If an early processing defect limits liberation of cholesterol, that event should precede downstream changes attributable to insufficient product availability.

If cholesterol liberation remains intact but its transfer fails, tracer experiments should distinguish product retention from inadequate initial hydrolysis.

If a downstream export lesion produces secondary lysosomal dysfunction, the export-related abnormality should appear before the later impairment in fresh substrate processing.

The mitochondrial branch generates separate predictions.

If altered lipid distribution contributes to MAO dysfunction, the relevant membrane state and enzymatic change must be measured together.

If direct RUBV mitochondrial remodeling independently changes MAO, restoring lysosomal throughput alone may not correct the phenotype.

If MAO remains functionally normal despite substantial mitochondrial structural changes, the proposed MAO-specific bottleneck does not explain the measured effect in that system.

This is where the model becomes testable rather than merely descriptive.

It does not need every RUBV-induced abnormality to fit one explanation.

It requires the predicted abnormalities to occur in a defined sequence, produce measurable consequences, and respond to interventions aimed at the specific causal step.

17. The decisive experiments — how the causal chain can be established or rejected

A rigorous experiment must record failures as plainly as successes. Normal CE-to-free-cholesterol flux and normal downstream delivery during the predicted RUBV stage weaken Bottleneck One; normal isoform-specific MAO activity despite verified mitochondrial remodeling weakens Bottleneck Two; persistence of neural abnormalities after correcting the implicated defect weakens mediation. For proposed maternal routes, absence of a reproducible maternal intermediate or offspring association defeats that particular sequence. For childhood vaccination, the strong null epidemiology [8,9] sets a high burden for any prespecified exception.

The accumulated evidence identifies a concrete experimental program.

The purpose is not to demonstrate again that RUBV infects cells, changes their membranes, or causes developmental injury under congenital conditions.

Those observations already have substantial experimental or clinical foundations.

The decisive task is to determine whether Jacob's specified bottlenecks mediate neural injury in a susceptible biological setting.

17.1 Establish the infection stage and host background

A valid comparison must separate uninfected controls from clearly characterized RUBV-exposed systems.

It must document viral strain, infection burden, cell type, host genotype, developmental stage, and the maturation of viral replication structures.

Early entry should not be treated as equivalent to established infection with mature modified endolysosomal compartments.

Matched wild-type and attenuated-strain comparisons must account for differences in viral biology rather than assume equivalent exposures.

Genotype-related effects must be evaluated independently of differences in viability or overall viral burden.

17.2 Determine whether Bottleneck One occurs

The critical measurements are direct and quantitative: the rate of incoming cholesteryl-ester and triglyceride delivery to the lysosomal system; LAL localization, abundance, and catalytic activity; time-dependent disappearance of labeled cholesteryl ester and appearance of free cholesterol; lysosomal membrane lipid composition, calibrated acidity, and V-ATPase function; cholesterol accessibility immediately following hydrolysis; NPC2-related transport, lysosomal cholesterol residence, and downstream sterol arrival.

The experiment must identify the first measurable failure.

Substrate backlog with reduced initial hydrolysis supports a processing limitation.

Preserved hydrolysis with abnormal free-cholesterol retention supports a post-hydrolysis transfer defect.

Early export abnormalities followed by later acidification and hydrolysis defects support a feedback sequence.

Normal flux throughout the predicted infection stage would contradict the proposed lysosomal bottleneck in that system.

17.3 Determine whether Bottleneck Two occurs

The second investigation must directly measure mitochondrial outer-membrane conditions and MAO function.

The key endpoints are MAO-A catalytic activity, MAO-B catalytic activity, enzyme abundance, membrane association, outer-membrane lipid composition, and relevant mitochondrial physiological functions.

Measurements should occur at defined stages of RUBV infection.

A strong result would demonstrate a reproducible infection-associated alteration in one or both MAO isoforms that corresponds to a measurable membrane or protein-regulatory defect.

Restoring the identified defect should restore the relevant enzymatic function.

Mitochondrial remodeling without MAO dysfunction would establish a different outcome and require revision of the proposed second bottleneck.

17.4 Connect the biochemical defects to neural development

Establishing an intracellular abnormality is only one part of a disease mechanism.

The next experiment must determine whether the abnormality changes neural development.

Relevant systems include human microglia, neural progenitors, neurons, glial co-cultures, and developmentally characterized organoids.

Measurements should cover the affected cellular functions, neural maturation, and interactions among neural populations.

The strongest causal evidence would establish four linked observations: RUBV exposure precedes the cellular defect; the defect precedes a neural phenotype; correcting the specific defect restores the relevant neural function; and the relationship changes predictably with a defined susceptibility genotype.

A correction that merely reduces viral replication or prevents generalized cell death cannot independently establish that the proposed lipid-processing or MAO mechanism mediated the neural outcome.

17.5 Test the human susceptible-subset claim

The final level requires appropriately designed human evidence.

Confirmed exposure history, genetic characterization, timing, biochemical intermediates, and prospectively defined developmental outcomes must be evaluated together.

Prenatal wild-type infection must be distinguished from preconception maternal exposures and childhood vaccination.

A genotype-specific result requires independent replication and appropriately controlled comparisons.

A demonstrated cellular mechanism does not automatically establish the prevalence of that mechanism among autistic individuals.

Likewise, identifying a small biological subgroup does not establish the proposed cause of ASD unless the exposure, mediator, and clinical outcome are connected.

The decisive result is not merely the detection of RUBV-responsive molecules in an autistic individual. It is the demonstration of an exposure-dependent, mechanistically mediated developmental effect in a prospectively identifiable susceptible population.

18. The ten-paper integration — one model, multiple independent evidence streams

Jacob's research program developed through ten interconnected investigations. Their importance lies not in the number of papers but in the distinct scientific questions they bring together.

Paper I introduced the distinction between intracellular molecular presence and functional availability, particularly in lysosomal lipid processing and cholesterol transfer.

Paper II established the human congenital rubella-autistic phenotype evidence and brought developing neural tissue into the mechanistic investigation.

Paper III examined RUBV-responsive tryptophan and kynurenine-pathway regulation as an additional immune-metabolic route.

Paper IV addressed lipoprotein cargo, apolipoprotein-associated transport, receptor handling, and lysosomal delivery.

Paper V separated viral entry from the formation and maturation of RUBV replication compartments, making infection stage a necessary experimental variable.

Paper VI integrated the neurodevelopmental argument and specified the evidentiary requirements for disease-level causation.

Paper VII investigated direct RUBV mitochondrial effects and the lipid-sensitive MAO-A/MAO-B system.

Paper VIII examined the relationship between glial lipid recycling, specialized glycosphingolipid production, membrane organization, and neurodevelopment.

Paper IX concentrated on LAL-centered functional capacity and the consequences of reduced effective lipid throughput.

Paper X extended the acid-membrane-LAL interface, its biochemical constraints, and its proposed relationship to neural outcomes.

Together, these papers identify a coherent biological question: Can RUBV exposure disrupt the intracellular processing and distribution of molecules required for neural development in a host whose genetic background limits the ability to compensate?

The evidence streams are complementary, not identical. The strongest direct RUBV observations come from viral cell biology, human neural exposure experiments, lipid composition studies, and mitochondrial investigations. Independently, host functional studies establish the biological operations required for LAL processing, cholesterol transfer, oxysterol production, and membrane-sensitive MAO function.

Jacob's contribution is to identify where these findings could form a causal sequence and to specify the measurements necessary to distinguish that sequence from alternative explanations.

The model does not gain strength by treating all ten investigations as if they measured the same outcome.

It gains strength by combining their distinct findings into a set of predictions that can be tested within the same biological system.

19. Discussion — the importance of functional availability in rubella-associated neurodevelopmental injury

The investigation of rubella-associated neurodevelopmental injury spans more than fifty years.

The earliest compelling evidence came from children with congenital rubella. Subsequent experiments established viral replication architecture, interactions with host proteins, changes in lipid composition, and direct engagement of developing human neural tissue.

These observations have usually been investigated in separate experimental traditions.

Clinical researchers examined congenital injury and developmental outcomes. Virologists studied replication compartments and viral proteins. Biochemists examined membrane lipids and enzyme systems. Cell biologists investigated cholesterol trafficking, lysosomal acidification, and mitochondrial organization.

Jacob Boatman's model brings these observations together around a specific physical principle.

A molecule can be abundant within a cell yet fail to reach the process that requires it.

That distinction applies to lysosomal cargo, active enzymes, liberated cholesterol, and downstream lipid-dependent physiology.

It also creates measurable differences between otherwise similar cellular states.

Two cells may contain similar amounts of cholesterol while distributing that cholesterol differently. Two cells may express comparable quantities of LAL while processing incoming cholesteryl esters at different rates. Two mitochondria may contain similar amounts of MAO protein while displaying different catalytic activity because of differences in membrane conditions.

These distinctions matter especially during neural development, when cellular processes must operate in a coordinated, stage-dependent manner.

The archived experimental findings identify specific RUBV effects relevant to this investigation.

The virus modifies endolysosomal compartments. Its P150 protein interacts with ARL8A. Infection changes host phospholipid and fatty-acid composition. Rubella capsid associates with mitochondria and disrupts aspects of mitochondrial architecture and function. Human fetal-brain experiments demonstrate viral involvement in microglia and responses in neighboring neural populations.

None of these observations is an invented result of the integrated model.

The unresolved scientific issue is whether the functional interruptions identified by Jacob mediate the particular neural outcomes under investigation.

That question is sharpened by competing explanations.

RUBV-associated interferon responses, protein-translation disturbances, direct mitochondrial protein interactions, and other cellular changes could produce neural injury without the proposed LAL-MAO sequence.

An effective scientific investigation must determine which mechanisms are necessary, which are sufficient, which act independently, and which interact under specific host conditions.

The clinical exposure question requires the same discipline.

Historical congenital rubella findings demonstrate that infection during fetal development can produce autistic clinical syndromes. They do not automatically establish equivalent outcomes after childhood exposure or maternal preconception infection.

The large epidemiological studies of MMR and autism provide substantial contrary evidence for a vaccine-caused ASD mechanism. Their findings must remain part of the interpretation of the proposed childhood vaccine-strain route.

A defined susceptible-subset model should produce evidence strong enough to distinguish a genuine exposure-by-genotype effect from background genetic risk, correlated maternal and family factors, and chance subgroup associations.

The causal model is most valuable when it can fail under experimental pressure.

If RUBV replication proceeds without the predicted alteration in lipid processing, the first bottleneck must be reconsidered.

If mitochondrial membrane remodeling occurs without any relevant MAO functional change, the second bottleneck must be reconsidered.

If biochemical abnormalities occur without mediating a developmental phenotype, those abnormalities cannot be assigned responsibility for that phenotype merely because they accompany infection.

And if a proposed human exposure route does not produce the predicted genotype-dependent effect in well-controlled studies, that route cannot remain an established explanation.

Scientific strength comes from precise predictions that distinguish one mechanism from another—not from making an explanation broad enough to accommodate every possible result.

The integrated research program therefore presents both an explanation and a challenge.

It identifies a specific series of molecular events for investigation and defines the experiments capable of determining whether that series connects RUBV exposure with autistic neurodevelopmental outcomes.

20. Conclusion — the human outcome, the molecular intersections, and the decisive challenge

The evidence does not end at a shared molecular name. It begins with children affected by congenital rubella, continues into infected human developmental tissue and measured viral remodeling of lipids and organelles, and now yields a falsifiable two-bottleneck model. The experiment that matters will track exposure, susceptible genotype, lipid-processing and MAO function, and neural outcome in the same defined system. That result—not rhetoric—will determine whether the proposed molecular chain explains a rubella-associated autistic subgroup.

The first evidence came from children. Congenital rubella produced severe developmental injury, and investigators documented full and partial autistic syndromes in the affected population. Decades later, researchers directly observed RUBV in human fetal brain tissue and measured changes in neighboring neural cells. The history of this question begins with a human outcome and now reaches individual cells and molecules.

RUBV does not leave the host-cell environment untouched. Primary experiments demonstrate altered membrane lipid composition, endolysosomal replication structures, an interaction between P150 and ARL8A, capsid association with mitochondrial membranes, and interference with mitochondrial protein import. Independent human neurodevelopmental research identifies measurable abnormalities in some ASD-related populations involving sphingolipid species and protein-regulatory machinery. These findings do not originate in one laboratory or one experimental method. They give the causal investigation multiple concrete, independently measurable points of entry.

The key advance in Jacob Boatman's ten-paper program is not a new list of molecular names. **It is an explicit proposal for how cellular materials can be present while failing to become functionally available at the place and time that development requires them.** The first proposed bottleneck concerns LAL-centered lipid processing and the immediate handling of liberated cholesterol. The second concerns MAO-A and MAO-B

in their mitochondrial outer-membrane environment. The predicted result is a genotype-dependent disturbance in neural development following a defined RUBV exposure.

The ASD evidence deserves a central role in this investigation. Congenital rubella establishes the clinical precedent. Direct fetal-brain RUBV experiments identify a susceptible developmental environment. Human autistic-regression lipidomics and PABPC1-associated neurodevelopmental findings identify exact molecules and functional systems to compare—not just broad claims about inflammation or metabolism. The differences among these studies demand careful matching of cell type, developmental stage, biochemical species, and exposure history.

The decisive scientific challenge is direct: identify the susceptible host; document the RUBV exposure; capture the earliest impaired lipid-processing step; determine whether mitochondrial MAO activity changes; follow the effect into neural development; and rescue the relevant function by correcting the implicated mechanism. That sequence has the power to support, distinguish, or defeat the proposed causal routes. The congenital wild-type, maternal preconception, and childhood attenuated-strain questions must remain separate; negative population evidence concerning MMR cannot be erased by invoking an unspecified subset.

The human findings do not require researchers to assume that rubella explains every autistic person. Autism is heterogeneous. They require scientists to take seriously a documented infectious cause of neurodevelopmental injury with autistic presentations and to test whether Jacob's mechanism accounts for an identifiable subgroup.

The historical cases showed the outcome. Modern experiments identified the virus inside developing neural tissue. Biochemistry identified specific cellular changes. Jacob's work identifies a mechanism that can now be challenged at each step.

The next advance should not be another broad association. **It should be the experiment that follows rubella from exposure to a defined cellular interruption, from that interruption to neural development, and from the affected neural system to a clinically meaningful ASD-related outcome.**

That is the evidence this question demands—and the test this manuscript places before the field.

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