The Uncleared Signal

Alzheimer's disease as a disorder of lipid regulation — an evaluation of Estela Area-Gómez's programme: the contact site, the cholesterol sensor, and what the years since the theory was stated have returned

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A cell that has taken up too much cholesterol tells itself so by holding a short peptide in a membrane. Cutting that peptide is how the message ends. On this reading the enzyme at the centre of Alzheimer's disease is not a maker of poison but the machinery that clears a signal — and the disease is a homeostatic correction left running for fifty years.

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Abstract

A cell that has taken up too much cholesterol must tell itself so. The message travels from the plasma membrane inward, and it is delivered in a peculiar way: as a short peptide, held in the membrane of the endoplasmic reticulum, whose affinity for cholesterol gathers the lipid into a small rigid island. The island is a signalling platform. Enzymes assemble on it, esterify the excess sterol, damp down new synthesis, and restore the set point. Then the peptide is cut, the island disperses, and the message ends. The peptide is C99 — the fragment of the amyloid precursor protein left in the membrane after β-secretase, and the substrate from which γ-secretase makes amyloid-β. On this reading, the enzyme at the centre of Alzheimer's disease is not a producer of poison. It is the machinery that clears a signal.

This paper evaluates the body of work through which Estela Area-Gómez, with Eric Schon and a laboratory that has now run for nearly two decades, built that reading into a theory of the disease. The claim is not that lipids are involved in Alzheimer's disease; almost everyone concedes that. The claim is stronger and more specific: that the disease *is* a lipid disorder, that its lesion is located at a particular sub-domain of the endoplasmic reticulum where it touches the mitochondrion, and that plaques and tangles are downstream outputs of a homeostatic loop that has been left switched on.

Five load-bearing propositions organise the programme. **The amyloid machinery has an address.** Presenilin-1, presenilin-2, and γ-secretase activity are enriched not diffusely in the endoplasmic reticulum but in the mitochondria-associated ER membrane — a lipid-raft-like contact domain — and the long-standing "controversy" over presenilin localisation dissolves once one recognises that a protein concentrated at a contact reports into every fraction the contact touches. **The pathology is one of substrate, not product.** Enzyme function is a rate, not a level; measured as the ratio of product to available substrate, γ-secretase in Alzheimer's tissue is not overactive but *underactive*, and what accumulates first is C99. **C99 is a cholesterol sensor that builds its own domain.** It is not an inert precursor: it clusters cholesterol, nucleates the contact, drives sterol from the plasma membrane into the ER, and thereby amplifies the very platform on which it sits. **The amyloid-β 42:40 ratio is a readout of membrane geometry.** If the raft in which cleavage occurs is thinned by the incorporation of kinked, unsaturated acyl chains, the substrate must tilt to preserve hydrophobic matching, and a tilted substrate is cut in the wrong register. **Familial and sporadic disease are one process with two entry lanes** — the fragment rises, or the cholesterol rises, and each raises the other.

The years since the theory was stated in this form have been unusually kind to parts of it and unkind to others, and this evaluation separates the two. On the credit side: the enzyme predicted in 2020 to thin the membrane has been identified and its activation by C99 demonstrated; the contact has been shown to be a regulator of cholesterol balance in *healthy* cells, which is what a

theory of dysregulation requires; the peripheral lipid signature the theory predicted has been found in serum from carriers of a fully penetrant mutation at ages six to twelve, roughly a decade before phosphorylated tau moves; a protective genetic variant has been shown to read out in the same lipid layer; and the bioenergetic claim has been sharpened from "less ATP" to a specific failure of fuel selection. On the debit side: nearly all the primary measurements of contact function are made in fibroblasts and cell lines rather than in neurons in situ; "increased MAM function" is a rate of lipid synthesis in a biochemical fraction and not a directly observed contact; an independent group reports the opposite directional effect for a closely related manipulation; the membrane-thickness explanation of the 42:40 ratio competes with a well-supported protein-intrinsic account and is the programme's most over-stated step; and the argument from therapeutic failure has been partly overtaken, since two anti-amyloid antibodies now produce small but real clinical effects.

The most consequential feature of the theory is one its own summaries under-sell. It is *specific about direction*. Contact function is elevated in Alzheimer's models and patient cells and depressed in α-synuclein mutants; a compound developed to *increase* contacts is a candidate therapy in amyotrophic lateral sclerosis and would be exactly wrong in Alzheimer's disease. A framework that says only "contact sites matter in neurodegeneration" explains nothing. One that assigns each disease a sign, and stakes treatment on it, can be wrong — which is the point.

1. Introduction: What Kind of Theory Is This?

1.1 A programme, not a hypothesis about plaques

The work evaluated here began with a question about where a protein lives. In 2009 Area-Gómez and colleagues reported that presenilin-1 and presenilin-2, and the γ-secretase activity they carry, are enriched in a subcompartment of the endoplasmic reticulum that lies in close apposition to mitochondria (Area-Gómez et al., 2009). That is a paper in subcellular fractionation. It reads as a technical correction to a confused literature, and for several years it was cited as one.

What grew from it is a general theory of Alzheimer's disease, stated with increasing confidence across a series of syntheses (Schon & Area-Gómez, 2010; Area-Gómez & Schon, 2016; Area-Gómez & Schon, 2017; Pera et al., 2020; Area-Gómez & Schon, 2024): that the disease is fundamentally a disorder of lipid regulation; that its lesion is a hyperactive organelle contact; and that the two lesions the field has organised itself around — the plaque and the tangle — are downstream consequences of that dysregulation rather than its cause.

The programme now spans the founding localisation work, functional assays of the contact in patient cells, a mechanistic account of what the C99 fragment does there, a lipidomic effort in human cohorts, and parallel investigations in Parkinson's disease, amyotrophic lateral sclerosis, Charcot-Marie-Tooth disease, and several rare lipid disorders. It has generated something close to a hundred papers. It is a research programme in the strict sense: a hard core of commitments, a protective belt of subsidiary claims, and a characteristic method — measure the rate of a lipid reaction in a patient's own cells, and let the number decide.

1.2 The reversal at the centre

Every theory of this disease has a move at its centre that determines everything downstream. In the amyloid cascade the move is that the aggregating peptide is the agent (Hardy & Higgins, 1992; Selkoe & Hardy, 2016). Here the move is different, and it is worth stating in its simplest form because everything else follows from it.

The efficiency of an enzyme is defined by the rate at which it converts substrate to product. If a cell produces three units of product where it once produced one, that looks like activation — unless the three units came from five units of substrate, in which case the enzyme has become *less* efficient, not more. The field measured amyloid-β, found more of it, and concluded that γ-secretase was

overactive. Area-Gómez's argument is that the field measured the wrong quantity: what should be measured is the ratio of amyloid-β to C99, and by that measure the enzyme in Alzheimer's tissue is impaired.

This is not merely a bookkeeping correction. It relocates the pathogenic species. If γ-secretase is failing, then the primary abnormality is the accumulation of its uncut substrate, and amyloid-β is a partial and misleading record of a cut that increasingly does not happen. The plaque becomes the residue of a process, read at the wrong end.

Two independent lines make the reversal more than an interpretive preference. Sun and colleagues expressed 138 pathogenic presenilin-1 mutations and measured what γ-secretase did with them: the great majority raised the ratio of amyloid-β42 to amyloid-β40 while *reducing* total amyloid-β production (Sun et al., 2017). And in monogenic Alzheimer's disease, a large isogenic panel of human induced-pluripotent-stem-cell neurons carrying mutations in either *APP* or *PSEN1* converged on a shared endosomal abnormality that was attributable to the β-secretase-derived carboxy-terminal fragments and *not* to amyloid-β (Kwart et al., 2019). The substrate, not the product, carried the phenotype.

1.3 What this evaluation asks

Three questions organise what follows.

What does the programme establish about the cell biology of this disease? Not what it asserts — what the laboratory work supports, considered independently of the framing that surrounds it.

Which of its stated predictions have since been tested? A theory that made checkable commitments in 2020 and can be audited against the record in 2026 is in a rare and enviable position, and the audit is the most informative thing one can do with it.

Where is it weakest, and what would refute it? These are stated in Sections 11 and 12, without softening.

Sections 2 through 9 assemble the account from the primary work and the independent literature that bears on it. Section 10 audits the theory's stated predictions against the record and grades the evidence. Section 13 draws the consequences for measurement and treatment.

2. The Address: Where the Amyloid Machinery Lives

2.1 A contact, not an organelle

The relevant structure has been known, in one form, since 1990, when Vance isolated from rat liver a membrane fraction that co-purified with mitochondria but was demonstrably endoplasmic reticulum, and that carried the enzymes of phospholipid synthesis (Vance, 1990). She called it fraction X. It is now called the mitochondria-associated ER membrane, or MAM: the region where the two organelles approach to within tens of nanometres without fusing, held apart at a fixed distance by protein tethers and exchanging lipid and calcium across the gap.

Two features of this structure matter for what follows. First, it is not a compartment but an *apposition* — a functional state of a membrane rather than a fixed piece of anatomy, formed and dispersed on demand. Second, it is a raft: a cholesterol- and sphingolipid-enriched, detergent-resistant, lipid-ordered domain, thicker and more rigid than the bilayer around it, in which enzymes with an affinity for that environment become concentrated and their activities modulated.

2.2 The presenilins are MAM proteins

Before 2009 the subcellular localisation of the presenilins was a standing embarrassment. They had been reported at the endoplasmic reticulum, the Golgi, the nuclear envelope, endosomes, lysosomes, the plasma membrane, and the mitochondria — a promiscuity that read more like an artefact of method than a fact of biology. Area-Gómez and colleagues attacked it with three independent approaches at once: subcellular fractionation, direct γ-secretase activity assay, and immunocytochemistry. All three converged. Presenilin-1, presenilin-2, and γ-secretase activity are highly enriched in the MAM (Area-Gómez et al., 2009).

The explanation for the prior confusion is contained in the result. A protein concentrated at a contact site will report into every fraction that the contact touches. Pin the localisation to the interface and the promiscuity dissolves.

This is the discovery on which the programme rests, and its implication is large. The catalytic core of γ-secretase — the enzyme whose mutations cause early-onset familial Alzheimer's disease — is a protein of an ER–mitochondrial contact. The mutations that cause the disease are mutations in a contact-site protein. And because γ-secretase is a raft-resident enzyme and the MAM is a raft-like

domain, the localisation is not incidental: it is where the chemistry prefers to occur.

2.3 The whole processing line

γ-secretase performs the second of the two cuts that liberate amyloid-β. If the MAM claim is to account for amyloidogenesis rather than only its final step, the earlier machinery ought to be there too. An independent group working in Nice found that it is. Del Prete and colleagues showed that the amyloid precursor protein and its proteolytic fragments are present in the MAM in cells expressing wild-type or familial-mutant precursor and in the brains of transgenic mice; that both β- and γ-secretase are present and enzymatically active there; and that cells carrying the Swedish familial mutation show increased ER–mitochondria contact together with increased accumulation of neutral lipids, reversible by inhibiting either secretase (Del Prete et al., 2017).

Two laboratories, different cities, different models, converging on the same address — and, importantly, tying the *act* of processing to a change in the contact's lipids. The amyloidogenic route is a contact-site event.

2.4 Function, not just location

Localisation is one claim; dysregulation is another. Area-Gómez and colleagues measured the two canonical readouts of contact function — cholesteryl-ester synthesis and phospholipid synthesis — and found both significantly increased in presenilin-mutant cells and, decisively, in fibroblasts from patients with *both* familial and sporadic Alzheimer's disease (Area-Gómez et al., 2012). The same paper established that the MAM is an intracellular detergent-resistant, raft-like domain, which is why the raft-preferring secretases are found there.

Note what that experiment is. It is not a stain, not a transcript, not a correlation with post-mortem pathology. It is a rate of lipid synthesis measured in a living cell taken from a living patient, elevated in the common sporadic form of the disease as well as the rare inherited one. Whatever else one concludes, that observation demands an explanation, and the amyloid cascade does not supply one.

A separate group supplied the human-tissue counterpart. Hedskog and colleagues examined ER–mitochondria contacts in Alzheimer brain and in mouse and neuronal models, reported upregulation of contact-associated proteins in the diseased brain, and — the load-bearing detail — found the upregulation present in the transgenic mouse *before* plaques deposited (Hedskog et al., 2013). If the change precedes the plaque, it is not a scar left by the plaque.

3. The Reversal: Substrate, Not Product

3.1 What is actually elevated

The programme's most disruptive empirical claim is that Alzheimer's tissue is defined less reliably by an excess of amyloid-β than by an excess of C99 relative to the amyloid-β made from it. Amyloid-β levels vary enormously between patients; the C99-to-amyloid ratio, on this account, does not.

The claim did not originate here, and Area-Gómez has never said it did. An independent literature had been converging on the fragment for a decade. Lauritzen and colleagues, in Checler's laboratory, showed that C99 rather than amyloid-β is the principal contributor to early intraneuronal lesions in triple-transgenic mouse hippocampus (Lauritzen et al., 2012), and later that intraneuronal aggregation of C99 induces amyloid-β-independent lysosomal and autophagic pathology (Lauritzen et al., 2016). The same group has since put the question in its sharpest form — whether γ-secretase should be understood as a *beneficial inactivating enzyme* for a toxic fragment rather than as a producer of a toxic peptide (Checler et al., 2021).

From a different direction, Nixon's laboratory identified a specific molecular crime committed by the fragment: the tyrosine-682-phosphorylated β-carboxy-terminal fragment of the precursor protein inhibits the vacuolar ATPase, and this — not amyloid-β — is what fails to acidify the autolysosome in Alzheimer and Down syndrome mouse models (Im et al., 2023), a failure that drives the autophagic build-up which those authors argue yields senile plaques from within dying neurons (Lee et al., 2022). Hung and Livesey independently showed that altered γ-secretase processing disrupts lysosome and autophagosome function in monogenic disease (Hung & Livesey, 2018).

Three laboratories, working on endosomes, lysosomes, and autophagy rather than on contact sites, arrived at the same defendant. That convergence is the strongest external support the substrate-reversal has, and it is largely independent of anything to do with the MAM. It is also still moving: the Nice group has most recently used a bimolecular fluorescence probe to visualise where C99 dimerises inside the cell and to separate the toxicity attributable to the dimer from that of the monomer (Badot et al., 2026), which matters here because the concentration-dependent self-association of the fragment is precisely what the structural work predicted would compete with its cholesterol binding (Song et al., 2013).

3.2 Loss of function, and whose loss

If C99 accumulates because it is not cut, then the familial mutations are loss-of-function mutations. This has been argued before, from other premises: De Strooper set out the loss-of-function reading of presenilin mutations (De Strooper, 2007), and Heilig, Xia, Shen, and Kelleher described a presenilin-1 mutation causing near-complete abolition of γ-secretase activity in a patient with cotton-wool-plaque Alzheimer's disease (Heilig et al., 2010). Sun's survey of 138 mutations gave the quantitative version (Sun et al., 2017).

Area-Gómez's version differs from the Shen–Kelleher "presenilin hypothesis" in an important way that is often blurred. That account locates the pathogenic consequence in the *loss of presenilin's physiological functions*. This account locates it in the *accumulation of the uncut substrate* — a gain of a lipid-active peptide, arriving by way of a loss of proteolysis. The distinction is testable and matters therapeutically: on one reading you must restore presenilin function, on the other you must lower C99, and they are not the same intervention.

3.3 The clinical pharmacology, read from the reversal

Two large trial failures are conventionally read as evidence against amyloid. Read through the substrate reversal, they say something more specific.

γ-Secretase inhibition with semagacestat did not merely fail; it made patients measurably worse (Doody et al., 2013). On the amyloid account this is an anomaly requiring an auxiliary explanation. On the substrate account it is the predicted result: inhibiting the enzyme that clears C99 raises C99.

The BACE-inhibitor failures are the harder case and the programme has always acknowledged it. Verubecestat, which lowers C99 by preventing its formation, also worsened cognition (Egan et al., 2018), and BACE inhibition produced rapid regional brain-volume reduction that was non-progressive and reversed on withdrawal (Sur et al., 2020). The theory's response — that some C99 production is required for a normal cellular function, so that suppressing it below the physiological range is itself harmful — is not an evasion; it is the natural consequence of holding that the fragment is a signalling intermediate rather than waste. But it converts an apparent prediction into a constraint: any therapy aimed at C99 must *normalise* it, not abolish it. Section 13 returns to this.

4. C99 Is a Cholesterol Sensor

4.1 The binding site

The proposal that the amyloid precursor protein senses cholesterol was not made first by this laboratory. Sanders's group, working by solution NMR on the C99 fragment itself, showed that its transmembrane domain is unusually flexible and that it binds cholesterol directly, with a defined binding surface just upstream of the γ-secretase cleavage site; they had raised the sensor question in structural terms several years earlier (Beel et al., 2008; Beel et al., 2010; Barrett et al., 2012). Binding is in competition with C99 self-association (Song et al., 2013), which is itself relevant: at high concentration the fragment prefers itself to the sterol.

That is the structural warrant. The functional claim built on it is the programme's own.

4.2 The peptide that builds its own platform

Montesinos and colleagues asked what C99 does at the contact and answered that it behaves as a lipid-sensing peptide that *makes* the domain it occupies. Because of its affinity for cholesterol, C99 delivered to the ER for cleavage clusters cholesterol into transient detergent-resistant domains — that is, it nucleates MAM. When C99 accumulates, it drives the internalisation of extracellular cholesterol and its trafficking from the plasma membrane to the ER, expanding these regulatory domains, and, as the homeostatic consequence, inducing cholesterol esterification while attenuating de novo synthesis (Montesinos et al., 2020).

This closes a circle that had been open since 2012. The "upregulated MAM" measured in patient fibroblasts is not an unexplained observation; it is what an accumulating cholesterol-clustering peptide would produce. And the loop is self-amplifying: C99 builds the platform, the platform is where C99 would be cleaved, and the failure to cleave it keeps the platform standing.

The same sequence has been reproduced *in vivo* under a different insult. In a controlled-cortical-impact model of traumatic brain injury — one of the better-established environmental risk factors for later dementia — injured cortex and hippocampus showed increased C99 together with increased contact-site activity measured three ways: phospholipid synthesis, sphingomyelinase activity, and cholesterol turnover, with cell-type-specific changes in microglial lipid composition (Agrawal et al., 2023). The model is not confined to genetically engineered cells.

4.3 The loop stated plainly

Assembled, the physiological cycle the theory proposes runs as follows.

1. Cholesterol accumulates in the plasma membrane beyond a set point.
2. The precursor protein is internalised in cholesterol-rich endosomes; β-secretase, active at low pH, cuts it to C99.
3. C99 arrives at the ER, where its cholesterol-binding domain gathers sterol into a raft — the MAM.
4. On that platform, cholesterol-handling enzymes are recruited and activated: esterification proceeds, synthesis is suppressed, the excess is disposed of into lipid droplets.
5. γ-secretase cuts C99. The platform disperses. The signal ends.

Alzheimer's disease, on this account, is step 5 failing. The signal is never cleared, so the correction never stops. The cell continues importing cholesterol to the ER in response to an alarm that has already been answered, and the chronic operation of an acute homeostatic mechanism becomes the disease.

That is a specific and unusual kind of pathogenesis: not a toxin, not an aggregate, not a deficiency, but a control loop stuck in the on position. It has the virtue of explaining why the disease takes decades — a loop stuck on does its damage by accumulation of consequence, not by acute injury.

4.4 The physiological version, which the theory needed

A theory of dysregulation requires a regulation to dysregulate. Until recently the strongest statement of the contact's normal role in cholesterol balance was inferential. Montesinos and colleagues then addressed it directly, showing that scavenger-receptor-B1-mediated uptake of cholesterol from high-density lipoprotein stimulates formation of MAM domains, which in turn suppress the de novo cholesterol biosynthetic machinery — proposing the contact as a regulatory hub for cellular cholesterol homeostasis in its own right (Montesinos et al., 2024, preprint).

This is arguably the most important development in the programme since 2020, and it is easy to overlook because it is not about Alzheimer's disease at all. It converts the MAM from a structure that behaves oddly in a disease into a normal control node whose set point can be pushed. Only the second kind of structure can support a theory of the first kind. It also sets the therapeutic rule stated in Section 13: an intervention that abolishes the contact abolishes a physiological regulator.

5. The Thickness of a Membrane

5.1 Hydrophobic matching and the tilt

The most audacious proposition in the programme concerns the number the field treats as its most specific molecular signature: the ratio of amyloid-β42 to amyloid-β40.

γ-Secretase is an intramembrane protease that is not sequence-specific. It engages its substrate and cuts processively, in roughly tripeptide steps, releasing a distribution of peptide lengths. Where in that distribution the products fall depends on the geometry of the enzyme–substrate engagement. The proposal is that this geometry is set, in part, by the thickness of the membrane in which cleavage occurs. In a raft of normal thickness, the transmembrane helix of C99 matches the bilayer and is cut predominantly to amyloid-β40. If the raft is *thinner*, the helix must tilt to maintain hydrophobic matching; the tilt changes its alignment with presenilin-1; and the cut falls in a different register, favouring the longer peptide.

The consequence, stated without hedging in the theory's own summaries, is that the elevated 42:40 ratio in Alzheimer's disease is fundamentally *a surrogate marker for the thickness of a membrane*. The field's most-cited molecular index of the disease would be, on this reading, a ruler rather than a poison.

5.2 The named enzymes

What would thin a raft? Membrane thickness in a lipid-ordered domain tracks the length and straightness of the acyl chains in its phospholipids. Saturated chains are long and straight; unsaturated chains are kinked and effectively shorter. So an enzyme that preferentially loads unsaturated chains into the phospholipids of the domain will thin it.

The theory named two candidates, both resident at the contact. ACAT1 (gene *SOAT1*), the cholesterol-esterifying enzyme, favours oleate. And acyl-CoA synthetase long-chain family member 4, ACSL4 (historically FACL4), preferentially activates the polyunsaturated fatty acids C20:4 and C20:5. Activate the raft, and by this route you thin it.

This is a real prediction with named molecules, and it was on the record.

5.3 The experiment that tested it

In 2025 the laboratory delivered the corresponding experiment. Montesinos and colleagues report that MAM formation enhances ACSL4 activity, promoting activation of arachidonic acid and its preferential incorporation into phosphatidylcholine through the Lands cycle in concert with the contact-resident acyltransferase LPCAT4; that elevated C99 induces contact remodelling through cholesterol clustering, which activates ACSL4 and alters phosphatidylcholine composition; and that the effect is mirrored in Alzheimer models and in fibroblasts, neurons, and immune cells derived from both familial and sporadic patients (Montesinos et al., 2025, preprint).

Read against the 2020 statement, this is a prediction registered and then met: the named enzyme, the named fatty-acid preference, the named consequence for the phospholipid composition of the domain, and — importantly — an extension of the measurement from fibroblasts into neurons and immune cells. It is currently a preprint and must be graded as such. But the structure of the claim is exactly what one wants from a theory: it said in advance which enzyme to look at, and the enzyme was there.

Independently, and from a laboratory with no stake in the MAM framework, Dawkins and colleagues in Steiner's group showed that remodelling membrane lipids modulates γ-secretase *processivity* (Dawkins et al., 2023) — that is, that the lipid environment does in fact shift the length distribution of the products. The general principle underlying the thickness claim has external support.

5.4 Where the claim is weakest

It is nonetheless the programme's most over-stated step, and this evaluation says so plainly.

The dominant explanation for the 42:40 shift is protein-intrinsic. Chávez-Gutiérrez's group showed that Alzheimer's-causing mutations shift amyloid-β length by destabilising the interactions between γ-secretase and its successive intermediates (Szaruga et al., 2017), and that the amyloid-β profiles generated by presenilin-1 variants predict pathogenicity and age at onset (Petit et al., 2022). Structural biology has since visualised the engagement directly: cryo-electron-microscopic structures of human γ-secretase bound to the amyloid precursor protein and to Notch, and of the substrate-recognition and cleavage mechanism, describe how the substrate is captured and stepped through the active site (Yang et al., 2019; Zhou et al., 2019; Guo et al., 2024).

Nothing in that literature requires a lipid explanation, and nothing in it excludes one. Processivity and bilayer thickness are not competing mechanisms; the former is the proximate description and the latter a proposed modulator of it, and Dawkins's result shows the modulation is real. But the theory's own summaries present the thickness account as though it were the explanation rather than a contribution to one, and the quantitative question — how much of the observed ratio shift in human disease is attributable to membrane geometry — has not been

answered by anyone. Until it is, "the 42:40 ratio is a surrogate for membrane thickness" should be read as a hypothesis with a mechanism, not as an established fact.

6. One Disease, Two Entry Lanes

6.1 The unification claim

The theory holds that familial and sporadic Alzheimer's disease are the same disease reached by two routes. In the familial form, mutations in the precursor protein or the presenilins raise C99 directly; C99 then drives cholesterol into the ER. In the sporadic form, cholesterol delivery to the ER rises first, for any of several reasons, and the elevated cholesterol raises C99. Either way the contact is upregulated, and the downstream consequences are common.

This is a stronger claim than it appears. It predicts that sporadic tissue should show elevated C99 despite normal *APP* and *PSEN* genes — which is what is reported — and it predicts that the sporadic risk architecture should be enriched for genes that move cholesterol.

6.2 ApoE4 as a lipid lesion at the contact

The programme's account of the major sporadic risk factor is mechanistically specific. ApoE4-containing lipoproteins are recycled from the endolysosomal system less efficiently than ApoE3, cholesterol accumulates intracellularly, and C99 rises. The direct test was done in 2016: Tambini and colleagues showed that ER–mitochondrial communication and contact function, measured as phospholipid and cholesteryl-ester synthesis, are significantly increased in cells treated with ApoE4-containing astrocyte-conditioned medium compared with ApoE3 — and, tellingly, that the effect required lipoprotein-enriched preparations and was not produced by lipid-free ApoE protein (Tambini et al., 2016). The pathogenic entity is the particle, not the apolipoprotein in isolation.

The wider field has moved substantially toward a lipid reading of ApoE4 since, without in most cases adopting the MAM frame. ApoE4 disrupts intracellular lipid homeostasis in human iPSC-derived glia (Sienski et al., 2021); it impairs myelination through cholesterol dysregulation in oligodendrocytes (Blanchard et al., 2022); it causes lipid accumulation that degrades microglial surveillance of network activity (Victor et al., 2022); and homozygous *APOE4* is linked to damaging lipid droplets in human Alzheimer microglia (Haney et al., 2024), against a background in which lipid-droplet-accumulating microglia are established as a dysfunctional, pro-inflammatory state of the ageing brain (Marschallinger et al., 2020). Mahley, from outside the programme, has explicitly argued that ApoE4 targets mitochondria and the MAM complex in neuropathology including Alzheimer's disease (Mahley, 2023).

This convergence is the strongest circumstantial case the theory has. It did not persuade the field; the field arrived on its own, from genetics and single-cell biology, at a lipid-centred account of the dominant risk gene.

6.3 The regional question, partly answered

The 2020 statement of the theory listed regional vulnerability among the features it could not explain. That admission was creditable and it is now partly out of date, by the programme's own work.

Transcriptomic and respirometric analysis of aged *APOE*-targeted-replacement mice showed that the entorhinal cortex — the region that fails first in human disease — is differentially affected by ApoE4 with respect to bioenergetics, with regulation there that differs from an Alzheimer-resistant region (Area-Gómez et al., 2020). A companion targeted lipidomic study found the entorhinal cortex more susceptible than the primary visual cortex to ApoE4-associated lipid alterations, with dose-dependent elevation of ceramides, glycosylated sphingolipids, and bis(monoacylglycero)phosphate — species that accumulate when endosomal-lysosomal handling fails (Miranda et al., 2022).

That does not explain selective vulnerability. It does show that the lipid lesion is regionally graded in the direction the disease takes, which is the first thing such an explanation would need.

6.4 The genetic audit, a decade on

The 2020 argument reviewed fifteen risk genes with coding-region variants and claimed eleven were consistent with altered cholesterol trafficking or contact-site function. Genome-wide analysis has since expanded the map by an order of magnitude, identifying dozens of risk loci (Bellenguez et al., 2022), and rare-variant work has implicated *ABCA1* — a cholesterol exporter — alongside *ATP8B4*, a lipid flippase (Holstege et al., 2022).

The honest reading of the expanded genetics is mixed. Lipid and endolysosomal genes are unambiguously over-represented, and *ABCA1*, *ABCA7*, *SORL1*, and *APOE* all sit comfortably in the theory's frame. But the largest single signal in the modern architecture is immune, and much of it is microglial. A framework that assigned eleven of fifteen genes to its own mechanism in 2020 cannot claim the same fraction of a much larger set today without straining. The programme's response has been to follow the immune arm into lipid — the microglial lipid-droplet work above, and its own finding that loss of nuclear TDP-43 drives triglyceride accumulation, lipid droplets, phagocytic activation, and interleukin-1β release in human microglia-like cells (Kabra et al., 2025, preprint) — which is a reasonable move, but it is expansion under pressure, not a prediction fulfilled.

7. Downstream: Lipids, Energy, and Tau

7.1 Sphingolipids and the respiratory supercomplexes

The clearest mechanistic chain in the programme runs from the fragment to the energy budget, and it is a lipid chain from end to end.

Pera and colleagues showed that C99, in addition to its endosomal pool, is present at the MAM, where it is normally cleaved rapidly; that in Alzheimer cell models unprocessed C99 accumulates there; that this drives elevated sphingolipid turnover and alters the lipid composition of both the contact and the mitochondrial membranes; and that this altered composition interferes with the assembly and activity of the mitochondrial respiratory supercomplexes (Pera et al., 2017).

The last step is what makes the chain matter. The respiratory chain does not float as independent complexes; its components assemble into higher-order structures whose formation depends on the lipid environment of the inner membrane. Change the lipids and the chain assembles badly. Bioenergetic failure in Alzheimer's disease, on this account, is not a primary mitochondrial defect at all: it is a lipid defect read out in a mitochondrion.

7.2 Not running out of gas

The programme's most useful conceptual intervention outside Alzheimer's disease proper is a critique of how the field has thought about mitochondria in neurodegeneration. In a review with Guardia-Laguarta, Schon, and Przedborski, the argument is made that reduced oxidative phosphorylation in these disorders has been too readily assumed to mean an energy crisis that kills the neuron — a connection never actually demonstrated — and that the mitochondrion should be read instead as an organelle sitting at the junction of many metabolic pathways rather than as a battery running flat (Area-Gómez et al., 2019).

The positive version of that argument arrived in 2025. Larrea and colleagues showed, in familial models of amyotrophic lateral sclerosis, that impaired ER–mitochondrial crosstalk *impedes the use of glucose-derived pyruvate as mitochondrial fuel, forcing a shift to fatty acids to sustain energy production*, and that over time this alters electron flow and the active/dormant status of complex I in spinal cord but not brain (Larrea et al., 2025).

This is a materially different claim from "less ATP". It says the contact governs *fuel selection*, that its failure forces a substrate switch, and that the switch has tissue-specific consequences. It is also a better fit to what is actually observed in the Alzheimer brain — an early, regional decline in glucose

utilisation that long precedes cell loss — than any account in which mitochondria are simply weaker.

7.3 Cholesterol and tau

A theory that demotes both plaques and tangles owes an account of tau. The programme's is indirect and rests substantially on work from another laboratory. Van der Kant and colleagues showed in patient-derived iPSC neurons that cholesterol metabolism is a druggable axis regulating tau and amyloid-β *independently*; that promoting cholesterol efflux by non-esterification routes reduced tau phosphorylation; and that the protective effect of statins on tau pathology depended on cholesterol esterification (van der Kant et al., 2019). Esterification is an ACAT1 reaction and ACAT1 is a contact-site enzyme, so the axis runs through the structure at issue.

Supporting this, β-secretase inhibition — but not γ-secretase inhibition — lowers phosphorylated tau in familial neurons, which points at the fragment rather than at the peptide. And ACAT1 blockade has an independent literature in Alzheimer models, where it enhances neuronal autophagy and reduces mutant tau content at a presymptomatic stage (Shibuya et al., 2015) and stimulates lysosomal proteolysis in microglia (Shibuya et al., 2014). Notably, acute ACAT1 blockade has been shown to *increase* MAM cholesterol and strengthen ER–mitochondria connectivity (Harned et al., 2023) — a result that complicates any simple "block ACAT1 to fix the contact" therapeutic story, and which Section 13 takes seriously.

The tau arm remains the weakest link in the downstream chain. It is a real mechanism with real pharmacology, but it is a link the programme borrows rather than one it built.

7.4 Calcium, the second current

The contact carries two currents, and the second is calcium. Presenilin-2 — specifically, and not presenilin-1 — modulates calcium shuttling between the organelles, and familial mutants strongly favour transfer by increasing the physical apposition of the membranes rather than by acting on the uptake machinery (Zampese et al., 2011). Nanomolar amyloid-β raises expression of the channels that carry the transfer, increases contact number, and elevates mitochondrial calcium (Hedskog et al., 2013).

The laboratory's most recent contribution here identifies a new component of the machinery: STIM1, the ER calcium sensor known for store-operated entry at the plasma membrane, also interacts with the mitochondrial partners PTPIP51 and GRP75 at the contact, and lowering it disrupts ER-to-mitochondria calcium transfer, reduces basal mitochondrial calcium, impairs maximal respiration, and lowers ATP production; the interaction is conformation-dependent and maps to a defined segment of the protein (Orantos-Aguilera et al., 2026). This is basic contact-site cell biology rather than an Alzheimer's result, but it matters for the theory: it strengthens the case that the structure is a genuine, molecularly defined regulatory node with a tunable calcium function,

not a fractionation artefact.

8. The Peripheral Reading: Lipids in Blood

8.1 The prediction, on the record

The theory's diagnostic prediction was stated explicitly and in advance: if the disease is a lipid disorder driven by contact-site dysregulation, then specific lipid species should be altered in predictable ways, and those alterations should be detectable in accessible tissue — serum for the lipids, blood mononuclear cells for the fragment. It was further stated that prior "agnostic shotgun" lipidomics had failed to deliver a usable diagnostic precisely because it was not mechanism-guided, and that a mechanism-based panel should do better.

This is the kind of commitment on which a theory can be judged.

8.2 A signature at age six

The test was run in the largest and best-characterised autosomal-dominant kindred in the world: the Colombian *PSEN1*-E280A families, in whom the genetic outcome is certain and the age at onset is narrowly predictable, and in whom the ordinary biomarker sequence has been mapped in detail over fifteen years (Reiman et al., 2012; Fleisher et al., 2012).

In collaboration with Cardona-Gómez, Lopera, Villegas, Quiroz, Schon and others, the laboratory reports a serum lipidomic signature that distinguishes *asymptomatic* mutation carriers aged 6 to 40 from non-carriers with an area under the curve of 80–90 per cent, comparable to its performance in symptomatic carriers and in sporadic cases; latent-profile analysis resolves lipid states associated with risk and with resilience, shaped by genotype, sex, and *APOE* isoform; and the age-dependent dysregulation is in sphingolipid and glycolipid metabolism, validated against enzyme activity, glial phenotyping, and single-nucleus transcriptomics in post-mortem brain. The temporal claim is the striking one: ganglioside clearance deficits emerge by ages 6–12, pro-inflammatory shifts from age 13, and phosphorylated tau-217 elevation by age 20, with greater burden in female carriers and in *APOE4* carriers (Cardona-Gómez et al., 2025, preprint; extended in Cardona-Gómez et al., 2026, preprint).

If this holds, it is the most consequential result the programme has produced. It would place a lipid abnormality roughly a decade ahead of the earliest established fluid biomarker of the disease, in children, in a population destined with near-certainty to develop it. It would also convert the theory's central metaphysical claim — that the lipid lesion is upstream — from an argument about

mechanism into an argument about dates.

Three cautions are mandatory. These reports remain preprints and have not completed peer review. The design is cross-sectional across age bands rather than longitudinal within individuals, so "emerges by age 6–12" describes a group difference at that age, not a trajectory followed in a child. And *PSEN1*-E280A is a fully penetrant mutation in a gene the theory holds to act directly on the fragment; a signature in this kindred does not establish that the same signature precedes sporadic disease, which is where a preventive test would have to work. The sporadic comparison reported in the same work is a necessary start, not a substitute.

8.3 Resilience read in the same layer

The Colombian kindred also contains the field's most-studied protected individuals, and the laboratory has now read them in lipid.

The APOE3-Christchurch variant came to attention through a homozygous carrier of the *PSEN1* mutation who remained cognitively intact into her seventies despite an extraordinary amyloid burden (Arboleda-Velasquez et al., 2019), with a distinctive tau distribution and cellular profile at autopsy (Sepulveda-Falla et al., 2022), and heterozygosity for the variant has since been associated with later onset across the kindred (Quiroz et al., 2024). Working with post-mortem cortex from *PSEN1*-E280A carriers including Christchurch carriers, the group reports that familial and sporadic Alzheimer brains show extensive remodelling of lipid pathways — depletion of structural phospholipids, marked sphingolipid alterations — while Christchurch carriers show reduced cholesterol and phospholipid content, *preservation of ceramide pools*, enrichment of specific ganglioside fractions, increased sphingomyelinase activity, and coordinated downregulation of sphingolipid biosynthetic and remodelling genes, with cell-type-specific glial differences (Salomón-Cruz et al., 2026, preprint).

The structural logic here is worth naming, because it is what makes the result more than another lipid association. A theory that says a disease is a lipid dysregulation predicts that a protective genotype should show a *lipid* phenotype — not merely less pathology, but a different disposition of the same molecules. That is what is reported. Whether the lipid state is the mechanism of protection or a consequence of it is exactly the question this design cannot settle, and the paper is a preprint. But the prediction was of the right shape, and it was not obvious.

8.4 What it would take to be a biomarker

An AUC of 80–90 per cent in a genetically defined kindred is a demonstration of biological signal, not a clinical test. Three things stand between the two. The signature must be shown to be prospective within individuals rather than cross-sectional across ages. It must be shown in sporadic populations at a stage where intervention is conceivable. And it must be shown to add information beyond the plasma phosphorylated-tau assays that have advanced rapidly in the same kindred over the same period — a comparison the theory would welcome, because its claim is precisely that it moves earlier, and earliness is measurable.

9. The Same Lesion Elsewhere

9.1 α-Synuclein, and the sign reverses

The laboratory's parallel Parkinson's work is not a digression; it is the source of the framework's single most important discriminating property.

Wild-type α-synuclein is not, as had been supposed, a mitochondrial protein: it localises to the MAM. Pathogenic point mutations *reduce* its association with the contact, coincident with reduced ER–mitochondria apposition, decreased contact function, and increased mitochondrial fragmentation (Guardia-Laguarta et al., 2014). More recent work in post-mortem substantia nigra, in multiple-system-atrophy striatum, and in iPSC-derived neurons finds region- and disease-specific lipid changes, particularly in phosphatidylserine species, in the areas most affected (Barbuti et al., 2025), with the broader lipid case for synucleinopathy set out separately (Area-Gómez et al., 2025).

The point to hold is the sign. Contact function is *up* in Alzheimer's models and patient cells and *down* in mutant α-synuclein. A framework asserting only that "contact sites matter in neurodegeneration" would be unfalsifiable and useless. This one assigns a direction to each disease, which is a commitment that can be wrong.

9.2 TDP-43, cholesterol, and the microglial lipid droplet

The most active current expansion is into TDP-43 proteinopathy, and it bears on Alzheimer's disease more than it first appears, because TDP-43 co-pathology is present in a large fraction of Alzheimer brains.

In a knock-in mouse, TDP-43 dysfunction alters brain lipid pathways, drives lipid-droplet accumulation in brain and in fibroblasts, and links to myelin abnormalities — cholesterol being the bulk of myelin lipid — with corresponding lipid-droplet marker elevation in human post-mortem frontal cortex (García-Toledo et al., 2025). In human monocyte-derived microglia-like cells, knockdown of *TARDBP* suppresses cholesterol biosynthesis, upregulates fatty-acid uptake, accumulates lipid droplets, enhances phagocytosis, and raises interleukin-1β — and inhibiting diacylglycerol acyltransferase reverses the droplet formation, the phagocytosis, and the cytokine release, in patient-derived cells as well as knockdowns (Kabra et al., 2025, preprint). A companion review sets out cholesterol dyshomeostasis as a shared axis across neurodegenerative conditions with the contact as its regulatory platform (Fernàndez-Bernal et al., 2025).

Two things are notable. The lipid-droplet microglial phenotype reached here from TDP-43 is the same phenotype reached by others from *APOE4* and from ageing — a convergence that is now hard to dismiss. And the intervention that reverses it is a licensed class of enzyme inhibitor.

9.3 A contact-site disease family

Beyond the three major proteinopathies the programme has assembled, largely through collaborations, a set of monogenic disorders in which the lesion is at the contact: Charcot-Marie-Tooth type 2A, where mitofusin-2 mutations alter contact function in patient fibroblasts in proportion to disease severity while leaving respiratory-chain function intact (Larrea et al., 2019); hypomyelinating leukodystrophy, where the sphingolipid desaturase DEGS1 proves to be a contact-resident enzyme whose loss disrupts all four core contact functions (Planas-Serra et al., 2023); a complex hereditary spastic paraplegia from *RINT1* deficiency with defective lipid-droplet biogenesis and an inhibited Lands cycle (Launay et al., 2023); juvenile CLN3 disease reframed as a lysosomal cholesterol storage disorder (Chen et al., 2023); and, outside the nervous system, therapy-resistant neuroblastoma, where resistant tumour cells show reduced contacts and where it is specifically reduced *ceramide* synthesis and transfer, not calcium transfer, that confers resistance to apoptosis (Çoku et al., 2022).

The Charcot-Marie-Tooth result deserves emphasis because it dissociates two things the field routinely conflates: contact function was impaired and correlated with severity, while respiration was unimpaired. That is a clean demonstration that "mitochondria-associated" pathology is not the same as bioenergetic failure.

9.4 The generality problem, stated

The obvious objection follows immediately. If contact-site dysfunction appears in Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal degeneration, Charcot-Marie-Tooth, leukodystrophy, spastic paraplegia, neuronal ceroid lipofuscinosis, traumatic brain injury, alcohol exposure, coenzyme Q10 deficiency, and drug-resistant neuroblastoma — what is it about the contact that makes any of these the disease it is?

The programme has an answer with three parts, and it is stronger than the objection allows. The *direction* differs: up in Alzheimer's, down in mutant α-synuclein and in resistant neuroblastoma. The *driver* differs: an uncleaved sensing peptide, a mislocalised synuclein, a nuclear RNA-binding protein lost from the nucleus, a mutant tether. And the *lipid class primarily deranged* differs: cholesterol and sphingolipid in Alzheimer's, phosphatidylserine in synucleinopathy, triglyceride and cholesterol in TDP-43 proteinopathy.

Table 1 — The lesion, signed. What the framework claims for each disorder it has entered. The value of the table is that every cell in it is a commitment that could turn out to be wrong.

Disorder	Driver	Contact function	Lipid class chiefly deranged	Principal source
Alzheimer's disease	Uncleaved C99	Increased	Cholesterol, sphingolipid	Area-Gómez 2012; Pera 2017; Montesinos 2020
Traumatic brain injury	Injury-induced C99	Increased	Cholesterol, sphingomyelin	Agrawal 2023
Synucleinopathy	Mutant α-synuclein, mislocalised	Decreased	Phosphatidylserine	Guardia-Laguarta 2014; Barbuti 2025
ALS / TDP-43 proteinopathy	Nuclear TDP-43 loss	Decreased	Triglyceride, cholesterol	Larrea 2025; García-Toledo 2025
CMT type 2A	Mutant mitofusin-2	Altered, severity-graded	Phospholipid	Larrea 2019
Leukodystrophy (DEGS1)	Loss of a contact-resident desaturase	Disrupted	Sphingolipid	Planas-Serra 2023
Spastic paraplegia (RINT1)	Loss of ER–Golgi trafficking protein	Disrupted	Neutral lipid, phospholipid	Launay 2023
Resistant neuroblastoma	Selection under therapy	Decreased	Ceramide	Çoku 2022

That is a real answer. It is not yet a complete one, because it does not explain why a common downstream platform yields entorhinal cortex in one disease and spinal motor neurons in another, and Section 11 lists this among the theory's outstanding debts. But the framework is doing more work than "everything is contact sites" — it is assigning each disease a signed, driver-specific, lipid-specific lesion, and those assignments are checkable.

10. The Audit and the Ledger

10.1 Predictions on the record, checked

A theory that made specific commitments and can now be audited against the record is in an unusual position, and the audit is more informative than any amount of further argument. Table 2 sets out the commitments as they were stated, and what the intervening work returned.

Table 2 — The theory's stated commitments, audited. "Met" does not mean established beyond dispute; it means the claim was made in advance, the corresponding experiment was subsequently done, and the result went the predicted way.

Commitment as stated	What has since been reported	Verdict
Specific lipid species will be altered in predictable ways	Sphingolipid and glycolipid dysregulation in serum and post-mortem cortex of mutation carriers	Met, preprint
Those alterations will be detectable in blood	Serum signature separating asymptomatic carriers from non-carriers, AUC 80–90%	Met, preprint
An enzyme with a preference for C20:4/C20:5 will thin the raft	ACSL4 shown to be activated downstream of C99-driven contact remodelling; arachidonate loaded into phosphatidylcholine with LPCAT4	Met, preprint
The lipid signal will be early	Ganglioside clearance deficits at ages 6–12, ahead of p-tau217 elevation at 20	Met, preprint; cross-sectional
Contact function is a physiological regulator, not only a disease phenomenon	Contact formation driven by SR-B1/HDL uptake shown to suppress de novo cholesterol synthesis	Met, preprint
C99, not amyloid-β, carries the cellular phenotype	Independent isogenic iPSC panel attributes endosomal pathology to β-CTFs, not Aβ	Met, published, external
γ-Secretase is impaired, not overactive, in disease	138 PSEN1 mutations raise the 42:40 ratio while lowering total Aβ	Met, published, external
The 42:40 ratio is a surrogate for membrane thickness	Lipid remodelling shown to modulate processivity; no apportionment of the human shift attempted	Partly met; unquantified

Commitment as stated	What has since been reported	Verdict
Sporadic risk genes will concentrate in cholesterol handling	Lipid and endolysosomal genes over-represented, but the largest modern signal is immune	Partly met; strained
Regional vulnerability is unexplained	Entorhinal cortex shown preferentially susceptible to ApoE4 bioenergetic and lipid alterations	Moved, not solved
Sex difference is unexplained	Greater lipid burden reported in female carriers	Moved, not solved
Brain specificity is unexplained	No advance	Open
Therapies aimed at amyloid have all failed	Two antibodies now show small but significant clinical effects	Overtaken

The pattern is worth naming. Where the theory made a *biochemical* commitment, it has generally been met. Where it made an *epidemiological or clinical* commitment, it has fared less well. And a striking proportion of the affirmative evidence from the last two years sits in preprints that have not completed review, which is the single largest qualification on everything above.

10.2 The validity ledger

The claims are graded here on three tiers by the strength of the evidence behind them, not by how central they are to the theory.

Tier I — Established. Presenilins and γ-secretase activity are enriched at the ER–mitochondrial contact. The contact is a raft-like, detergent-resistant domain carrying phospholipid synthesis, cholesterol esterification, and calcium transfer. C99 binds cholesterol through a defined transmembrane surface. Contact-associated lipid-synthesis rates are elevated in fibroblasts from familial and sporadic patients. C99 accumulates in Alzheimer tissue and models and carries cellular phenotypes independently of amyloid-β. Most familial presenilin mutations reduce total amyloid-β production while raising the 42:40 ratio. Wild-type α-synuclein is a contact-site protein, and pathogenic mutants dissociate from it.

Tier II — Strong but incomplete. C99 nucleates the contact and drives plasma-membrane cholesterol to the ER. Contact function is upregulated *in the Alzheimer brain*, as opposed to in patient fibroblasts. ApoE4-containing lipoproteins raise contact activity. Elevated C99 at the contact drives sphingolipid turnover and impairs respiratory supercomplex assembly. Contact dysfunction shifts mitochondrial fuel selection from pyruvate to fatty acid. Cholesterol esterification regulates tau. Contact activity governs cholesterol homeostasis in healthy cells. C99-driven remodelling activates ACSL4 and alters phosphatidylcholine composition.

Tier III — Plausible, contested, or extrapolated. That the 42:40 ratio is fundamentally a readout of bilayer thickness. That contact upregulation is the *initiating* event in sporadic disease rather than an early consequence. That the serum lipid signature in a fully penetrant kindred generalises to sporadic disease. That the lipid state of Christchurch carriers is the mechanism of their protection rather than a correlate of it. That plaques and tangles are, in the strong sense, downstream outputs of contact dysregulation. That the same lesion accounts for the regional and cell-type selectivity of the disease.

A note on preprint dependence. Five of the results this evaluation treats as important — the cholesterol-homeostasis role of the contact, the ACSL4 mechanism, the serum lipidome, its extension, and the Christchurch lipid phenotype — are preprints. They are consistent with each other and with the published body of work, and they come from a laboratory with a long peer-reviewed record on the same questions. None of that substitutes for review. Any reader weighting this evaluation should discount those five accordingly, and note that if they were removed the audit above would look considerably thinner.

11. Where the Programme Is Weak

11.1 The fibroblast problem

The single most important limitation is that the foundational functional measurements — increased phospholipid synthesis, increased cholesteryl-ester synthesis, increased apposition — were made predominantly in fibroblasts and immortalised cell lines. Fibroblasts from patients are an excellent system: they carry the patient's genome, they are alive, and they give a rate rather than a snapshot. They are also not neurons, they do not have the geometry or the energetic constraints of neurons, and they do not develop the disease.

The programme has been extending into neurons steadily — iPSC-derived neurons in the C99 cholesterol-trafficking work, neurons and immune cells in the ACSL4 work, mouse cortex in the traumatic-injury and *APOE* studies, and post-mortem human cortex in the Colombian lipidomics. The trajectory is right. But the central quantitative claim of the theory — that contact function is upregulated in Alzheimer's disease — still rests most heavily on a non-neuronal cell.

11.2 What "increased MAM function" is measuring

The phrase does a great deal of work and is under-specified in most summaries. Operationally, it is the rate of transfer of phosphatidylserine from ER to mitochondria and its conversion to phosphatidylethanolamine, and the rate of cholesteryl-ester formation, measured with radiolabelled precursors in a subcellular fraction obtained by density-gradient centrifugation.

Three consequences follow. The measurement is of a biochemical activity, not of a physical contact; the two are related by inference. The fraction is an operational definition, and what appears in it depends on how the gradient is run. And an increase in the rate of a reaction could reflect more platform, more enzyme, more substrate, or a change in the environment that raises catalytic efficiency — the theory says the first, but the assay does not, by itself, distinguish them.

None of this is a defect peculiar to this laboratory; it is the state of the art for the structure. It is a reason to hold the quantitative claims more loosely than the narrative around them does.

11.3 The directional dissent

An independent group has produced results that cut against the simplest form of the model. Filadi and colleagues, in Pizzo's laboratory, argue that presenilin-2 modulates ER–mitochondria coupling by tuning the antagonistic effect of mitofusin-2 (Filadi et al., 2016), and have shown that expressing the presenilin-2 loop domain *loosens* coupling (Rossini et al., 2021). More directly awkward: Leal and colleagues, in the same laboratory that reported increased contacts in Alzheimer brain, found that knocking down mitofusin-2 *increases* ER–mitochondria contact and *decreases* amyloid-β production (Leal et al., 2016) — the opposite of the association the model would predict if more contact meant more amyloidogenesis.

The manipulations are not identical to the disease state, and a knockdown that changes contact number by a non-physiological route need not reproduce what an accumulating cholesterol-binding peptide does. But the tension is real and the field has not resolved it; recent reviews of contact sites in brain ageing and neurodegeneration reflect the unsettled state rather than a consensus (Azarnia Tehran & Pizzo, 2025; Guo et al., 2026). A reader should not be told this literature points uniformly in one direction.

11.4 The membrane-thickness step

Restated from Section 5.4 because it belongs in a list of weaknesses: the claim that the amyloid-β42:40 ratio is fundamentally a surrogate for bilayer thickness is presented in the theory's summaries with a confidence the evidence does not carry. Lipid remodelling demonstrably modulates γ-secretase processivity (Dawkins et al., 2023), and the thickness mechanism is plausible and specific. But the mainstream account of the ratio shift is protein-intrinsic and well supported structurally and kinetically, and no one has apportioned the observed human shift between the two. This is the step at which the programme most often over-reaches.

11.5 The therapeutic argument has been partly overtaken

The 2020 statement of the theory leaned on the total failure of amyloid-directed therapy. That premise no longer holds in the form it was stated. Lecanemab and donanemab both produced statistically significant slowing of decline in phase-3 trials in early Alzheimer's disease (van Dyck et al., 2023; Sims et al., 2023). The effects are small, purchased with amyloid-related imaging abnormalities, and their clinical meaningfulness is genuinely disputed — but "all trials aimed at amyloid have failed" is not an available premise in 2026.

The theory is not refuted by this. Its own text argued that amyloid-β is toxic and that removing it should benefit patients even if it is a secondary participant, which is close to a description of what the antibodies deliver: a real but modest effect from removing a downstream product. That is a creditable prior position. What it loses is the rhetorical force of the argument from total failure, and any restatement of the theory that continues to lean on that argument should be discounted

accordingly.

11.6 What remains unexplained

The 2020 statement listed, admirably, what it could not explain: early loss of olfaction; the brain-specificity of a phenotype produced by a ubiquitous mechanism; regional and cell-type vulnerability; the excess of affected women; and risk loci that do not fit.

Two of these have moved. Regional vulnerability has a first foothold in the *APOE* entorhinal work (Area-Gómez et al., 2020; Miranda et al., 2022). Sex has entered the data rather than the discussion, with a greater lipid burden reported in female carriers (Cardona-Gómez et al., 2025, preprint). Mixed pathology has moved too, via the TDP-43 arm.

Three have not. Brain specificity remains the deepest problem: the contact is universal, the enzymes are universal, patient fibroblasts show the lesion — and the patient's fibroblasts are fine. The theory needs an account of what the neuron does with a mis-set lipid platform that a fibroblast does not, and the obvious candidates — the extreme membrane demands of synaptic transmission, the length of the axon, the impossibility of dilution by division in a post-mitotic cell — are gestured at rather than demonstrated. Cell-type vulnerability within the brain is in the same position. And the fit of the modern genetic architecture is looser than the 2020 audit suggested.

12. What Would Refute It

A theory of this scope should say what would sink it. These follow from the claims as stated.

A direct measurement of contact function in Alzheimer's neurons that finds it normal or reduced. The central quantitative claim is directional. Contact-site apposition and lipid-transfer rates measured in identified neurons from human tissue or well-validated neuronal models, showing no elevation or an elevation only after tau pathology, would remove the theory's foundation. This is technically achievable now.

Serum lipid signatures that fail to precede phosphorylated tau in a longitudinal design. The temporal claim in the Colombian data is cross-sectional. Followed within individuals, if the lipid change tracks or lags plasma phosphorylated tau rather than leading it, the "upstream" claim fails on the only evidence that could establish it in humans.

A demonstration that C99 accumulation is downstream of another primary event. The fragment's priority is inferred from models. If in human tissue C99 elevation is shown to require prior tau pathology, prior inflammation, or prior lysosomal failure arising independently, the sensor is a passenger.

A normalisation of contact function that leaves the disease unchanged. With a tool compound now available to modulate contacts pharmacologically (Etxebeste-Mitxeltorena et al., 2025), the experiment is becoming feasible: bring contact activity back to baseline in a model with elevated C99, and if lipid composition, bioenergetics, and pathology are unchanged, the contact is a marker and not a mechanism.

Apportionment of the amyloid-β42:40 ratio to protein-intrinsic mechanisms alone. If careful reconstitution assigns the disease-associated ratio shift wholly to destabilised enzyme–substrate interactions with no residual attributable to bilayer geometry, the thickness claim falls, though the wider theory would survive it.

A cholesterol-normalising intervention, delivered early and demonstrated to hit the target, that fails to alter the course of disease. This is the therapeutic refutation and the slowest to obtain, and it requires an intervention that provably normalises the ER regulatory pool rather than merely lowering serum cholesterol — a distinction the statin trials never achieved.

13. Consequences for Measurement and Treatment

13.1 The two ratios nobody measures

The theory's most immediately actionable proposal is also its cheapest. It holds that the diagnostic quantity is not the level of amyloid-β but two ratios: amyloid-β42 to amyloid-β40, which the field already measures, and *total amyloid-β to C99*, which it essentially does not.

The second ratio is the direct index of whether γ-secretase is keeping up with its substrate. If the theory is right, it should separate disease from normal ageing better than either quantity alone, and it should move earlier. The proposal is to measure it in peripheral blood mononuclear cells, which are accessible, and the reagent problem the theory itself identified — that many antibodies raised against amyloid-β cannot distinguish it from the fragment that contains it — is the reason the measurement has not been routine. That is a solvable assay problem, not a conceptual one, and it is the single most informative experiment the theory asks for that no one has run at scale.

13.2 The first rule: tune, do not sever

Every plausible intervention here is bidirectional, and the programme's own results establish why.

The contact is a physiological regulator of cholesterol balance (Montesinos et al., 2024, preprint); abolishing it removes a control system. Suppressing C99 formation below the physiological range worsened patients (Egan et al., 2018; Sur et al., 2020). Blocking γ-secretase, which clears the fragment, worsened them badly (Doody et al., 2013). Acute ACAT1 blockade increases contact cholesterol and strengthens connectivity (Harned et al., 2023) — that is, a drug proposed for its downstream benefit pushes the upstream structure in the direction the theory calls pathogenic. And the one brain-permeable contact-modulating tool compound so far developed was selected to *increase* contacts, because in amyotrophic lateral sclerosis they are reduced (Etxebeste-Mitxeltorena et al., 2025); in Alzheimer's disease, on this theory, it would be precisely the wrong drug.

The rule that falls out is that the target is a set point, not a quantity to be minimised, and that any programme here needs a pharmacodynamic readout of the set point before it needs a molecule.

13.3 The candidate handles

Ordered by how directly they engage the mechanism.

ACSL4 and LPCAT4. The newly identified route by which contact formation loads arachidonic acid into phosphatidylcholine (Montesinos et al., 2025, preprint) is the most mechanistically specific node the theory has produced, and the one that would test the thickness claim therapeutically rather than descriptively.

ACAT1/SOAT1. The best-precedented handle, with independent efficacy signals in Alzheimer models on tau content, autophagy, and microglial proteolysis (Shibuya et al., 2014; Shibuya et al., 2015) and an established connection to the cholesterol–tau axis (van der Kant et al., 2019) — complicated, as above, by its effect on contact cholesterol.

Diacylglycerol acyltransferase. Inhibition reverses microglial lipid-droplet accumulation, phagocytic activation, and interleukin-1β release in patient-derived cells (Kabra et al., 2025, preprint). This is the arm that connects the lipid theory to the immune genetics, and the pharmacology is comparatively mature.

Sphingomyelinases. Contact-localised sphingomyelinase activity is elevated and drives the ceramide arm (Pera et al., 2017; Agrawal et al., 2023). Inhibitors exist; specificity is the problem.

Contact modulators directly. A biosensor-driven screen has produced a brain-permeable compound that modulates contacts and restores cholesterol trafficking in an ALS patient-derived model (Etxebeste-Mitxeltorena et al., 2025). For Alzheimer's disease the requirement is a compound of the opposite sign, and its existence would be the cleanest possible test of Section 12's fourth refutation condition.

13.4 Why timing is the whole therapeutic argument

If a lipid signature is detectable in carriers in the first decade of life while the corresponding clinical event is fifty years away, then the therapeutic implication is not a treatment for dementia. It is an argument that the window in which a lipid intervention could matter opens decades before any current trial enrols, and closes long before diagnosis.

That is an uncomfortable conclusion, because it is nearly untestable by the means the field currently uses. It also happens to be the conclusion the theory has been pointing at since it first claimed that the plaques were late. Whether or not the mechanism survives, this is the part of the argument most worth taking seriously: it says the field has been measuring the right disease at the wrong time.

14. Conclusion

The programme evaluated here has done something unusual. It took the least-examined object in the field's central pathway — the fragment that is cut, rather than the peptide that is released — and asked what it is *for*. The answer it gives is that the fragment is a sensor, that cutting it is the act of clearing a signal, and that the disease is what happens when a homeostatic message is left standing for fifty years.

The strongest parts of the account are the ones that were checkable and got checked. The amyloid machinery does have an address, and it is a contact site. Contact function is measurably elevated in cells taken from patients with both forms of the disease. The fragment does bind cholesterol, does build the domain it sits in, and does drive sterol into the endoplasmic reticulum. The enzyme predicted to thin the membrane was named in advance and has now been shown to be activated by the fragment. The contact regulates cholesterol balance in healthy cells, which is what makes the disease version a dysregulation. And a peripheral lipid signature, predicted as a consequence of all this, has been found in serum from carriers in childhood.

The weakest parts are also clear. The core measurements are made in the wrong cell. "Increased contact function" is a rate in a fraction, not a contact observed. An independent group finds the opposite directional effect for a related manipulation. The claim that the field's central molecular ratio is a measure of membrane thickness is the most striking thing the theory says and the least established. And the brain-specificity problem — a universal mechanism producing a disease of one organ, in a patient whose fibroblasts carry the lesion and are well — is not solved, or seriously attacked.

What should be taken from it, independent of whether the framework as a whole prevails, is a reorientation that no longer depends on it. Lipid handling is not a peripheral feature of Alzheimer's disease. The dominant risk gene is a lipid-transport gene whose pathology is now read, by several independent laboratories, as a lipid-storage phenotype in glia. Cholesterol esterification regulates tau. Membrane composition sets the length of the peptide the field has spent forty years measuring. Fuel selection, not fuel supply, is what fails in the mitochondrion. Whatever the fate of the contact-site hypothesis, that reorientation has already happened, and this programme did much of the work of making it happen.

The theory's own account of what it is doing is worth ending on, because it is honest about its status. It lists what it explains, and then it lists what it does not, and it declines to shoehorn the remainder in. Research programmes that survive tend to be the ones that keep that list.

Acknowledgments and Methodological Note

This is an evaluation of a published body of work, assembled from the primary literature. All cited references were verified against PubMed records at the time of writing. Where findings are currently available only as preprints they are labelled as such in the text and in the reference list, and the arguments that rest on them are qualified accordingly. Statements about what the programme predicted in advance are taken from its own published statements of the theory — principally the 2017, 2020, and 2024 syntheses listed below — and, where a prediction was set out at greater length there than in the published record, from an unpublished December 2020 document in which the authors stated the theory in full. That document is used only as the authors' own summary of their position, never as evidence for it; every empirical claim in this evaluation rests on a cited, publicly available source.

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