The Narrow Gate

TOMM40, Mitochondrial Protein Import, and the Architecture of Risk in Alzheimer's Disease

A Single Channel Through Which the Whole Mitochondrial Proteome Must Pass — and a Single Locus at the Most Confounded Address in the Alzheimer Genome

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A Dissertation Prepared under the ONS Methodology. A focused monograph deepening the TOM40 treatment of the Bioenergetic Collapse thesis (third volume of the Collapse trilogy) into a self-contained argument about the mitochondrial import gate and its gene.

“More than ninety-nine per cent of the mitochondrial proteome is made elsewhere and must be let in. Everything the organelle is, it imports — and it imports almost all of it through one door.”

— ONS Methodology Working Notes, 2026

For Allen Roses, who insisted that a gene next to APOE might still have something of its own to say — and for everyone still trying to tell the two neighbours apart.

A FOCUSED MONOGRAPH

Deepening §4.3 of Bioenergetic Collapse —

"The Gateway: TOM40 and Mitochondrial Protein Import" —

into a single integrated argument about one gene and one channel.

Where the Bioenergetic Collapse thesis treats TOM40 as one node in a network of mitochondrial failure, this monograph isolates the node and follows it in both directions at once: outward to the molecular machine that amyloid-β obstructs, and inward to the contested genetics of the TOMM40 locus and its poly-T polymorphism. The organising claim is that the channel's mechanistic importance and the locus's statistical independence are two different questions with two different answers.

Abstract

No locus in the human genome is at once so important to Alzheimer's disease and so difficult to interpret as the stretch of chromosome 19q13.32 that carries *APOE*, *TOMM40*, and *APOC1* in tight linkage disequilibrium. *APOE* is the strongest common genetic risk factor for late-onset Alzheimer's disease; *TOMM40*, its immediate neighbour, encodes the central β-barrel channel of the translocase of the outer mitochondrial membrane — the single pore through which more than ninety-nine per cent of the mitochondrial proteome must pass. This dissertation argues that *TOMM40* is uniquely positioned to expose a fault line that runs through the whole of Alzheimer genetics: the difficulty of separating a statistical signal from a mechanistic cause when two of the most consequential genes in the disease sit two thousand base pairs apart.

The thesis is constructed around a deliberate duality. **TOM40 the protein** is a molecular machine of exquisite importance and vulnerability: a nineteen-stranded β-barrel, resolved at near-atomic resolution in 2019, whose lumen imports every nuclear-encoded subunit of the electron transport chain, every tricarboxylic-acid-cycle enzyme, and the mitophagy sensor PINK1, and whose obstruction by amyloid-β — and, in Parkinson's disease, by α-synuclein at the partner receptor TOM20 — supplies a direct molecular mechanism by which proteinopathy throttles the bioenergetic machinery that proteinopathy itself degrades. **TOMM40 the locus** is a genetic object of an entirely different character: a poly-thymidine length polymorphism in intron 6 (rs10524523, "523") whose short, long, and very-long alleles segregate non-randomly with the *APOE* ε2/ε3/ε4 haplotypes, and whose reported power to predict the age at which late-onset disease begins has been claimed, contested, partially replicated, and built into a prospective prevention trial across fifteen years of argument.

Across ten chapters the dissertation traces the molecular machine (Chapters I–II), the genetic locus and its controversies (Chapters III–IV), the functional bridge from poly-T length to expression and import capacity (Chapter V), the preclinical neuroimaging and cognitive endophenotypes (Chapter VI), and the translational endpoint — the TOMMORROW trial, the first prospective test of a *TOMM40*-containing genetic-risk algorithm, in which pioglitazone failed to delay cognitive impairment but the genetic algorithm itself showed a three-fold enrichment of events that the trial's early termination left tantalisingly short of significance

(Chapter VII). The synthesis (Chapter VIII) reconciles the two faces of the gene: whether or not the poly-T tract carries independent statistical signal once *APOE* is conditioned out — a question the human data cannot yet settle — TOM40 the channel is a load-bearing node in the Mitochondrial Cascade Hypothesis, and a 2024 discovery of a transcriptional read-through chimera that tethers apoE to TOM40 at the mitochondrial surface suggests that the two genes may not be separable in mechanism even where they remain confounded in statistics. Seven falsifiable predictions are generated.

Keywords: TOMM40, TOM40, translocase of the outer membrane, mitochondrial protein import, β-barrel channel, poly-T polymorphism, rs10524523, APOE, linkage disequilibrium, mitochondrial cascade hypothesis, amyloid-β, ABAD/HSD17B10, Aβ–ABAD complex, AG18051, PINK1, TOMMORROW trial, age of onset, Alzheimer's disease.

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Chapter I — The Molecular Machine

1.1 The Problem of an Outsourced Proteome

The mitochondrion is the most consequential endosymbiont in the history of complex life, and the central fact of its biochemistry is one of dispossession. Over the roughly two billion years since the proto-mitochondrion was engulfed, the overwhelming majority of its ancestral genome was transferred to, or lost in favour of, the host nucleus. The human mitochondrial genome retains only thirteen protein-coding genes — all of them subunits of the oxidative-phosphorylation machinery — together with the ribosomal and transfer RNAs required to translate them. Every other constituent of the organelle, on the order of eleven hundred to fifteen hundred distinct proteins by current proteomic estimates, is encoded in the nucleus, translated on cytosolic ribosomes, and must then be delivered across one or both mitochondrial membranes to the compartment in which it functions.

This arrangement creates a logistical problem without parallel elsewhere in the cell. A protein destined for the mitochondrial matrix must cross two membranes; a protein destined for the inner membrane must cross the outer membrane and then be laterally inserted; a protein destined for the intermembrane space must cross the outer membrane and be trapped. The entire traffic — the continuous, lifelong importation of the organelle's working parts — converges on a small number of import machines embedded in the outer membrane, and at the centre of all of them stands a single channel. That channel is TOM40, the pore of the translocase of the outer membrane, and it is the subject of this dissertation.

The importance of this convergence cannot be overstated, because it makes the import apparatus a *single point of failure* for the organelle as a whole. A mitochondrion whose import is throttled cannot replace the subunits of its electron transport chain as they are oxidatively damaged; cannot import the matrix proteases and chaperones that constitute its internal quality-control system; and — as Chapter II will develop — cannot reliably import the very sensor, PINK1, that determines whether a failing mitochondrion is recognised and destroyed. The gate is narrow, and almost everything must pass through it.

1.2 Architecture of the TOM Complex

The translocase of the outer membrane is a multi-subunit assembly organised around the β-barrel channel TOM40 and a set of α-helical accessory subunits. Functionally these divide into three classes: receptors, the channel, and small regulatory subunits.

The **receptors** — TOM20, TOM22, and TOM70 — present the cytosolic face of the complex and perform the initial recognition of incoming preproteins. TOM20 recognises the amphipathic, positively charged N-terminal presequences that mark the largest class of matrix-destined proteins; TOM70, a tetratricopeptide-repeat receptor, recognises the internal targeting information of the hydrophobic carrier proteins of the inner membrane, often in concert with cytosolic chaperones of the Hsp70/Hsp90 families; and TOM22 serves both as a receptor and as the central organising subunit that bridges the cytosolic and intermembrane-space faces of the complex.

The **channel** is TOM40 itself, a member of the mitochondrial porin (VDAC-related) superfamily of β-barrel outer-membrane proteins. It forms the aqueous conduit through which unfolded and partially folded preproteins thread across the otherwise impermeable outer membrane.

The **small subunits** — TOM5, TOM6, and TOM7 — are single-pass α-helical proteins that nestle against the barrel and regulate the assembly, stability, and dynamics of the complex. TOM5 assists the transfer of preproteins from the receptors into the channel; TOM6 promotes assembly and stabilises the receptor-channel interaction; TOM7 has an antagonistic, destabilising role that renders the complex dynamic and, importantly, couples it to the downstream sorting-and-assembly machinery.

1.3 The β-Barrel and the Two Preprotein Paths

For three decades the architecture of the import gate was inferred from biochemistry, cross-linking, and low-resolution electron microscopy. That changed in 2019, when two groups independently determined the structure of the yeast TOM core complex by cryo-electron microscopy at near-atomic resolution (Araiso et al., 2019; Tucker & Park, 2019), building on the Neurospora structure of Bausewein and colleagues (2017). The resolved complex is a dimer: two TOM40 β-barrels, each formed of nineteen antiparallel β-strands, held together and tethered by two copies of TOM22 and a single structural phospholipid, with the small TOM subunits arranged around the periphery of each barrel.

The structural work resolved a long-standing functional puzzle: how a single, relatively narrow channel can accommodate the translocation of more than a thousand chemically diverse precursors. Araiso and colleagues described the lumen of each barrel not as a featureless tube but as a structured surface presenting distinct paths and exits, with the intermembrane-space domains of TOM40, TOM22, and TOM7 forming, at the centre of the dimer, a binding site that hands presequence-bearing preproteins to the downstream TIM23 translocase of the inner membrane. The barrel is, in other words, not a passive hole but an active gate whose interior chemistry guides different classes of substrate along different trajectories. Subsequent structures of the SAM-mediated assembly intermediate and of TOM complexes captured with bound preproteins have extended this picture into a near-complete mechanistic account of import (reviewed in Pfanner et al., 2019; and in the structural surveys of 2022).

The significance of the β-barrel fold for a dissertation on disease is twofold. First, the barrel is a *bottleneck of fixed and finite capacity*: import flux is a function of channel abundance and channel patency, both of which can be modulated in disease. Second, the barrel is *physically accessible from the cytosol* — a preprotein-conducting channel must, by definition, present an open mouth to the cytoplasm — which makes it a target for the very cytosolic aggregates whose accumulation defines neurodegeneration. Both points are developed in Chapter II.

1.4 Biogenesis: Importing the Importer

A deep curiosity attends the TOM complex: TOM40 is itself a nuclear-encoded protein that must be imported and assembled into the outer membrane, which raises the question of how the importer is itself imported. The answer reveals an elegant circularity. Newly synthesised TOM40 is recognised by the existing TOM complex, threaded into the intermembrane space, escorted by the small TIM chaperones, and then handed to the **sorting and assembly machinery** (the SAM/TOB complex), whose core component SAM50 is itself a β-barrel that catalyses the membrane insertion and barrel closure of nascent β-barrel clients, TOM40 among them. The mitochondrial import (MIM) complex assists in the assembly of the α-helical receptors.

This biogenetic pathway matters for the present argument because it establishes that TOM40 abundance is not a fixed constant but the output of a regulated assembly pipeline — one that depends on the prior existence of functional TOM and SAM complexes. A cell that begins to lose import capacity therefore faces a degenerative spiral: declining import compromises the delivery and assembly of the import machinery itself, so that the loss of the gate accelerates the loss of the gate. The relevance of any genetic variation that modulates *TOMM40* expression (Chapter V) must be read against this self-amplifying architecture.

1.5 Downstream Couplings and the Membrane Potential

The TOM complex is the common entry gate, but it is the first stage of a relay. Presequence-bearing matrix proteins are passed from TOM40 to the TIM23 complex of the inner membrane, whose import activity is driven by the electrical component of the proton-motive force (the membrane potential, $\Delta\Psi$) acting on the positively charged presequence, and completed by the matrix Hsp70 import motor that ratchets the polypeptide inward at the cost of ATP. Hydrophobic carrier proteins are passed instead to the TIM22 carrier translocase. Small intermembrane-space proteins with characteristic cysteine motifs are trapped by the mitochondrial intermembrane-space import and assembly (MIA) pathway via oxidative folding.

Two couplings deserve emphasis. First, import is **energetically expensive and energetically gated**: the presequence pathway requires both $\Delta\Psi$ and matrix ATP, so that a mitochondrion whose bioenergetics are already failing imports less

efficiently — another self-amplifying loop, in which energetic decline begets import decline begets energetic decline. Second, the dependence of TIM23 import on the membrane potential is precisely the property that the mitophagy sensor PINK1 exploits to report mitochondrial health, which makes the import relay not only a supply line but also a surveillance system. This dual role of the gate — supply and surveillance — is the hinge on which Chapter II turns.

1.6 The Gate as a Single Chokepoint

It is worth stating plainly the proposition that organises the molecular half of this thesis. Because the TOM complex is the obligatory entry point for the nuclear-encoded proteome, its function sits *upstream* of three distinct processes that a neuron must sustain indefinitely:

- **Replacement.** Every oxidatively damaged subunit of the electron transport chain, every spent TCA enzyme, every degraded matrix chaperone is replaced by a freshly imported copy. Import is the sole route of replacement; there is no alternative supply.
- **Surveillance.** PINK1, the sensor that flags depolarised mitochondria for destruction, is itself a TOM/TIM23 client. Its handling at the import machinery is the physical basis of mitochondrial quality control.
- **Disposal.** The decision to degrade a mitochondrion by mitophagy depends on PINK1 stabilisation, which depends in turn on the import status of the organelle. Import therefore gates not only what enters but what is destroyed.

A single channel that controls replacement, surveillance, and disposal is a chokepoint of extraordinary leverage. The remainder of this dissertation asks what happens when that chokepoint is obstructed (Chapter II), and whether inherited variation in the gene that builds it contributes to the risk and timing of Alzheimer's disease (Chapters III–VII).

Chapter II — Obstruction of the Gate

2.1 Amyloid-β at the Import Machinery: Two Mechanisms

The first and best-characterised assault on the import gate in Alzheimer's disease comes from amyloid-β itself, and it operates through two distinct and mutually compounding mechanisms that must be carefully distinguished.

The **transit-and-inhibit** mechanism was established by Hansson Petersen and colleagues (2008), who demonstrated that amyloid-β peptides are actively imported into mitochondria through the TOM machinery and come to rest in the mitochondrial cristae. Once inside the matrix and inner membrane, amyloid-β directly inhibits cytochrome c oxidase (Complex IV), reducing oxygen consumption and ATP output. This finding gave a concrete molecular mechanism to the Complex IV deficiency that had been observed in Alzheimer brain and platelets since Parker and colleagues' work in 1990, and it established the disquieting principle that the import gate is not merely vulnerable to amyloid-β but is the *route by which amyloid-β reaches the organelle's energetic core*.

The **obstruct-from-without** mechanism was established by Devi and colleagues (2006), who showed that amyloid precursor protein (APP) and amyloid-β accumulate *within* the TOM40 channel itself — lodged in the import pore from the cytosolic face — in the cortex of human Alzheimer's disease brain and in APP-transgenic mouse models. This physical obstruction blocks the import of nuclear-encoded Complex IV and Complex V subunits, degrading the delivery of the very proteins on which oxidative phosphorylation depends. Devi and Anandatheerthavarada (2010) extended the finding in a critical direction: not only is the pore obstructed, but TOM40 protein *abundance is itself reduced* in Alzheimer cortex, so that the disease depresses both the patency and the number of import channels.

The *mechanism* of this obstruction is more specific than passive lodging, and it answers the question of why a secretory protein should end up jammed in a channel built for mitochondrial preproteins. Anandatheerthavarada and colleagues (2003) showed that APP carries a cryptic, positively charged mitochondrial targeting signal

at its amino terminus that the TOM receptors recognise, so that a fraction of APP — specifically the non-glycosylated species that has not completed the secretory pathway — is mistargeted to the mitochondrion and begins to thread, amino-terminus first, into TOM40. Translocation then stalls: an acidic amino-acid domain in the body of APP cannot be drawn through the narrow TOM and TIM pores and acts as a stop-transfer signal, freezing the protein as a *transmembrane-arrested* intermediate in an "amino-terminus-in, carboxy-terminus-out" orientation, its cytosolic tail bearing the very amyloid-β sequence that downstream processing would release. The arrested protein forms stable ~480 kDa complexes with TOM40 and ~620 kDa super-complexes spanning TOM40 and TIM23, and the quantity accumulated scales with the severity of disease (Devi et al., 2006). The obstruction is therefore not a stray peptide plugging a hole but a stalled import intermediate jamming the gate — a distinction with two therapeutic corollaries taken up in Chapter VII: the plugging species is generated in proportion to APP load, and, being a stalled precursor, it is in principle a substrate for the cell's own import-stress clearance machinery.

The two mechanisms compound one another with a cruel logic. Amyloid-β that traverses the pore inhibits Complex IV from the matrix; amyloid-β that lodges within the pore degrades the import flux on which the matrix depends for its replacement subunits — including replacement Complex IV subunits. The mitochondrion is thus attacked simultaneously at its energetic core and at its supply line, and the supply line is exactly what it would need to repair the core.

2.2 Amyloid-β Inside the Matrix: ABAD as the Intramitochondrial Target

The transit-and-inhibit mechanism of §2.1 raises a question it does not answer: once amyloid-β has been imported through the TOM40 channel into the matrix, what does it bind? The most completely characterised answer is an enzyme that was, in fact, discovered *as* an amyloid-β-binding protein. In the mid-1990s the Columbia laboratory of Shi Du Yan and David Stern, screening brain libraries for intracellular partners of amyloid-β, recovered a mitochondrial-matrix enzyme of the short-chain dehydrogenase/reductase family — type 10 17β-hydroxysteroid dehydrogenase, encoded by the X-linked gene *HSD17B10* — and renamed it, for its newly found function, **amyloid-β binding alcohol dehydrogenase (ABAD)** (Yan et al., 1997). ABAD binds amyloid-β with low-nanomolar affinity at an interface overlapping its cofactor cleft, and the complex has been co-immunoprecipitated from Alzheimer brain but not from control tissue. The same enzyme has an essential, structurally separate role as a scaffold subunit of mitochondrial RNase P — the basis of the severe X-linked *HSD17B10* (HSD10) neurometabolic disease — which makes it both indispensable and, as the therapeutics below exploit, selectively targetable.

The pathological consequence of complex formation was established in the landmark report of Lustbader and colleagues (2004) in *Science*. Amyloid-β binding distorts the ABAD active site, and ABAD overexpression synergistically exacerbates amyloid-β toxicity — elevating mitochondrial reactive oxygen species, depressing membrane potential, impairing Complex IV, and increasing apoptotic markers beyond what either insult produces alone (Lustbader et al., 2004; Takuma et al., 2005). The Aβ–ABAD complex thereby supplies a second, enzyme-mediated route to the same Complex IV deficiency that §2.1 attributed to direct inhibition: imported amyloid-β throttles respiration both by acting on cytochrome c oxidase directly and by corrupting ABAD, whose oxidative output further sensitises the cyclophilin-D-gated mitochondrial permeability transition pore to calcium and drives cytochrome c release. On this evidence ABAD is the principal intramitochondrial effector of imported amyloid-β.

What lifts ABAD above a mechanistic footnote to the import story is that it is *druggable in a way the channel obstruction is not*. Lustbader and colleagues showed that a matrix-targeted, ABAD-derived decoy peptide (ABAD-DP) competitively displaces amyloid-β from ABAD and rescues the mitochondrial pathology in cells and

transgenic mice — the first proof that the complex can be therapeutically disrupted. The small-molecule probe AG18051 achieves the same end through an unusual mechanism: it forms a covalent adduct with the reduced nicotinamide of ABAD's NAD□ cofactor, allosterically destabilising the amyloid-β-binding loop and displacing the peptide, while leaving untouched ABAD's geometrically segregated scaffold role in mitochondrial RNase P — so that the catalytic and amyloid-binding functions can be ablated without incurring the lethal *HSD17B10*-deficiency phenotype (Kissinger et al., 2004; and the companion ONS pharmacology monograph on AG18051 and the Aβ–ABAD complex). The therapeutic principle is *toxic-complex disruption* rather than ligand clearance, and — crucially — it is reachable by small molecules that cross the mitochondrial double membrane where the antibody therapeutics directed at extracellular amyloid structurally cannot.

The relevance to a thesis on the import gate is that ABAD completes the arc of amyloid-β's assault on the mitochondrion, and closes it on a therapeutic note. Amyloid-β obstructs the gate from the cytosolic face (Devi); it transits the gate into the matrix (Hansson Petersen); and once inside it binds ABAD (Lustbader) — three lesions strung along a single import trajectory. The first two degrade the channel and remain, at present, pharmacologically intractable; the third converges on an enzyme whose amyloid-binding interface has an atomic-resolution inhibitor roadmap. The gate is thus not only a convergent *lesion*, onto which amyloid-β and α-synuclein funnel from different angles (§2.3), but a trajectory whose matrix endpoint is a convergent *target*.

2.3 The Parkinsonian Parallel: α-Synuclein at TOM20

That the import gate is a general target of proteinopathy, and not an Alzheimer-specific curiosity, was demonstrated by Di Maio and colleagues (2016), who showed that misfolded, oligomeric α-synuclein binds the import receptor TOM20, disrupts its interaction with its co-receptor TOM22, and thereby blocks the import of nuclear-encoded Complex I subunits in Parkinson's disease brain and in α-synuclein models. The mechanism is structurally parallel to the amyloid-β–TOM40 obstruction: in both diseases the defining proteinopathy converges on the mitochondrial import apparatus, and in both the resulting import deficit compounds direct electron-transport-chain inhibition by depleting the proteome from which damaged subunits would be replaced.

The convergence is mechanistically deep. Two different aggregation-prone proteins, associated with two different diseases, each find their way to the same machine and disable it from a different angle — amyloid-β at the TOM40 channel, α-synuclein at the TOM20 receptor. This is precisely the kind of convergence that the ONS methodology is designed to surface: distinct upstream pathologies funnelling onto a shared downstream node whose failure is common to both. The import gate is such a node.

2.4 apoE4 Fragments and the Mitochondrial Surface

A third assault on the mitochondrial import apparatus connects the molecular half of this thesis directly to its genetic half, and it is therefore of special importance. A fraction of apolipoprotein E escapes the secretory pathway into the cytosol of neurons, where it is cleaved by neuron-specific proteases into carboxyl-terminal-truncated fragments. Mahley, Huang, and colleagues demonstrated that these fragments — and the apoE4 isoform's fragments in particular — are neurotoxic and target mitochondria (Chang et al., 2005). Nakamura and colleagues (2009) showed that the apoE4 (1–272) fragment physically associates with mitochondrial proteins, among them components of the import machinery and the respiratory chain, and impairs mitochondrial function in neuronal cells.

The significance for this dissertation is that the protein product of *APOE* — the gene that sits two thousand base pairs from *TOMM40* — is itself a mitochondrial toxin whose isoform-specific fragments interfere with the organelle at, among other places, its import apparatus. The two neighbouring genes thus converge not only in the genome but on the same organelle and, plausibly, on the same machine. Chapter VIII returns to this convergence with the 2024 discovery of a transcriptional chimera that fuses the two gene products outright.

2.5 PINK1 Miscalibration and the Closed Loop

The deepest consequence of import obstruction is its effect on quality control. PINK1, the kinase that initiates Parkin-dependent mitophagy, is in healthy mitochondria continuously imported through TOM and TIM23 to the inner membrane, where the protease PARL cleaves it and targets it for degradation; PINK1 protein therefore never accumulates on a healthy organelle. When a mitochondrion loses its membrane potential, TIM23 import fails, PINK1 is no longer delivered to PARL, and it accumulates instead on the outer surface, where it phosphorylates ubiquitin and recruits Parkin — the canonical signal that consigns the organelle to destruction (Narendra et al., 2008, 2010).

The vulnerability is now apparent. Because PINK1's import — and hence its degradation — depends on the patency of the TOM40 channel, *obstruction of the channel by amyloid-β can stabilise PINK1 on the surface of a mitochondrion whose membrane potential remains intact*. The sensor is miscalibrated: it reports damage where there is only blockage, or fails to clear from organelles it should not be flagging. The same chokepoint that supplies the proteome and feeds replacement subunits to the respiratory chain also gates the sensor that decides which mitochondria die — and amyloid-β obstruction corrupts all three functions at once.

This closes a self-reinforcing loop that is, in the author's view, the single most important mechanistic claim that can be made about TOM40 in Alzheimer's disease. The upstream bioenergetic failure of the neuron (whatever its initial cause) generates and fails to clear amyloid-β; amyloid-β obstructs the import gate; obstruction of the gate degrades replacement, surveillance, and disposal; and the resulting accumulation of unrepaired, undisposed, failing mitochondria deepens the bioenergetic failure that began the cycle. The proteinopathy does not sit downstream as an inert signature; it feeds back to throttle the machine whose failure produced it.

2.6 Reading the Channel Two Ways

It will be useful, before turning to the genetics, to fix the two readings of TOM40 that the molecular evidence licenses. On the first reading, TOM40 is a **victim**: a channel obstructed and depleted by amyloid-β, its function collapsing as a downstream consequence of a proteinopathy whose origin lies elsewhere. On the second reading, TOM40 is a **driver**: a chokepoint whose finite capacity sets the bioenergetic ceiling of the neuron, such that any reduction in its abundance — by disease, or, the genetics will ask, by inheritance — lowers the threshold at which the cascade ignites. The two readings are not exclusive; indeed the closed loop of §2.5 requires both, since the channel must be both obstructed by the cascade and load-bearing within it for the feedback to operate. With this duality in hand we turn to the gene.

Chapter III — The Locus

3.1 The Most Confounded Neighbourhood in the Genome

To discuss the genetics of *TOMM40* is, unavoidably, to discuss the genetics of *APOE*, because the two genes are immediate neighbours on the long arm of chromosome 19 (band 19q13.32) and lie within a haplotype block of strong linkage disequilibrium that also encompasses *APOC1*. The genes are arranged head-to-tail across a span of a few tens of kilobases, with the 3′ end of *TOMM40* separated from the *APOE* transcription start by approximately two thousand base pairs. Within this block, alleles are inherited together far more often than recombination would predict; a marker in *TOMM40* is, to a substantial degree, a proxy for the *APOE* allele on the same chromosome, and vice versa.

This single fact of physical proximity is the source of every interpretive difficulty that follows. *APOE* carries the strongest common genetic risk factor for late-onset Alzheimer's disease — the ε4 allele, defined by two coding substitutions in the apoE protein — and any variant in tight linkage disequilibrium with ε4 will, by construction, show a statistical association with the disease whether or not it has any causal role. The central genetic question of this dissertation is therefore not "is *TOMM40* associated with Alzheimer's disease?" — it certainly is — but "does *TOMM40* carry signal that is *independent of* the *APOE* allele with which it travels?" The whole of Chapters III and IV is an attempt to do justice to that question.

3.2 The '523 Poly-T Polymorphism

The *TOMM40* variant at the centre of the genetic literature is not a coding change but a length polymorphism: a homopolymeric run of thymidine residues (a poly-T tract) located in intron 6 of the gene, catalogued as rs10524523 and referred to throughout the literature by the shorthand "523." The number of T residues in the tract varies between individuals from roughly fourteen to forty, and this length is heritable and stable.

The poly-T lengths are conventionally binned into allele classes. In the original scheme of Roses and colleagues the classes were **Short (S)**, with poly-T lengths up to about nineteen; **Long (L)**, roughly twenty to twenty-nine; and **Very Long (VL)**, thirty and above. Later work, recognising structure within the Long range, refined the binning — for instance into Short, Long-a, Long-b, and Very Long classes — and characterised the distribution of these lengths across human populations, finding substantial differences in poly-T length distribution between ancestral groups (Linnertz et al., 2012; Roses et al., 2010). The precise bin boundaries differ between studies, and this lack of a universal convention is itself a source of inconsistency in the replication literature, a point developed in Chapter IV.

3.3 Phasing: How Poly-T Length Tracks the APOE Alleles

The interpretive key to the entire poly-T literature is the non-random pairing of poly-T length with *APOE* allele on the same chromosome. In individuals of European ancestry the pattern is striking and consistent: the **Long** poly-T allele is almost always found on the chromosome that carries *APOE* **ε4**; the **Short** and **Very Long** alleles are found predominantly on chromosomes carrying *APOE* **ε3** (with the **Very Long** allele preferentially linked to ε3 and the Short allele distributed across ε3 and ε2 backgrounds).

This phasing has a crucial methodological consequence. Because L tracks ε4 so faithfully, any apparent association of the L allele with Alzheimer's disease in a general sample is almost entirely a restatement of the ε4 association — L is, in such samples, little more than a tag for ε4. The poly-T tract can only reveal *independent* signal in a setting where the *APOE* allele is held constant. This is the logic that led Roses and colleagues to their most important methodological move: the study of poly-T length within *APOE* ε3/ε3 homozygotes, in whom the ε4 effect is, by design, absent.

3.4 Roses' Age-of-Onset Hypothesis

The modern *TOMM40* literature begins with Allen Roses, who in 2010 reported that poly-T length predicts not merely the risk but the *age of onset* of late-onset Alzheimer's disease (Roses et al., 2010, *The Pharmacogenomics Journal*). The headline finding concerned *APOE* ε3/ε4 patients — heterozygotes carrying one ε3 and one ε4 chromosome — in whom the poly-T length on the ε3 chromosome could be examined while the ε4 chromosome was held in common. Among such patients who developed disease after sixty, those whose ε3 chromosome carried a *long* poly-T tract developed Alzheimer's disease on average some seven years earlier than those whose ε3 chromosome carried a *short* tract (on the order of 70 versus 77 years).

Roses framed this within an explicitly mechanistic hypothesis that connects the genetic locus to the molecular machine of Chapters I–II. The poly-T tract, he proposed, is not a neutral marker but a *cis*-regulatory element whose length modulates the expression of *TOMM40* (and possibly *APOE*), thereby tuning the abundance of the TOM40 import channel, and thus the mitochondrial import capacity, and thus — through the bioenergetic threshold logic of the Mitochondrial Cascade Hypothesis — the age at which an individual's declining import capacity crosses the pathogenic threshold. On this reading the poly-T length is a heritable setting of the bioenergetic ceiling, and the age of onset is the time required for age-related decline to lower a given ceiling to the floor.

This hypothesis is the high-water mark of the "TOMM40-as-driver" position. Its appeal is that it unifies the genetic and molecular halves of the story: the same channel that amyloid-β obstructs is the channel whose inherited abundance the poly-T tract sets. Its vulnerability — explored in the next chapter — is that the poly-T tract has never fully escaped the gravitational field of its *APOE* neighbour, and that the independent-signal claim has proven difficult to replicate.

3.5 The Mitochondrial Cascade Frame

Roses' hypothesis is a special case of a more general framework that this dissertation adopts as its interpretive backdrop: the Mitochondrial Cascade Hypothesis (MCH) of Swerdlow and Khan (2004; updated in Swerdlow, 2018). The MCH proposes that inherited mitochondrial function — set by maternally transmitted mitochondrial DNA and by nuclear-encoded mitochondrial genes — varies across the population and establishes a baseline bioenergetic capacity; that this capacity declines with age through the accumulation of somatic damage; and that disease-associated pathology emerges when a cell-type-specific bioenergetic threshold is crossed. On this view onset age is, to first order, a function of the *inherited starting point* rather than of any exogenous trigger.

TOMM40 is, within the MCH, an almost ideal candidate for a nuclear-encoded modifier of inherited bioenergetic capacity, precisely because TOM40 sets the import bottleneck. Whether the poly-T tract actually modulates that capacity in a way that survives statistical separation from *APOE* is the empirical question; but the *type* of gene that *TOMM40* is — a master regulator of import flux — is exactly the type the MCH predicts should matter. This congruence between a mechanistic framework and a candidate gene is part of what has kept the *TOMM40* hypothesis alive through fifteen years of contested replication. It is also, the sceptic will note, exactly the kind of congruence that can sustain a belief past the point at which the data warrant it. Chapter IV takes the sceptic's side.

Chapter IV — Driver or Passenger

4.1 The Confounding Problem, Stated Precisely

The difficulty of establishing an independent *TOMM40* effect is not a matter of insufficient data but of an inferential structure that more data alone cannot resolve. Because the poly-T tract is in strong linkage disequilibrium with *APOE*, the observed association of poly-T length with Alzheimer's disease in any unselected sample is a weighted sum of two contributions that the sample cannot separate: the genuine causal effect of *APOE* (largely ε4), transmitted to the correlated poly-T marker, and whatever genuine causal effect the poly-T tract may have in its own right. Standard association testing cannot apportion the signal between them, because the two variables are, in the population, largely redundant.

Three strategies have been used to attack the confound, each imperfect. The first is **stratification** — restricting analysis to a single *APOE* genotype, most powerfully ε3/ε3, so that the ε4 effect is held constant and any residual poly-T signal is, by construction, *APOE*-independent. The second is **conditional regression** — fitting the *APOE* genotype as a covariate and asking whether poly-T retains a significant coefficient. The third is **fine-mapping with ancestry** — exploiting the differing linkage-disequilibrium structure across populations to break the *TOMM40–APOE* correlation that holds so tightly in Europeans. Each strategy has yielded results, and they do not all point the same way.

4.2 The Case For Independence

The strongest evidence for an independent *TOMM40* effect comes from the stratified ε3/ε3 design, in which there is no ε4 to confound. Within ε3/ε3 homozygotes, several groups have reported that longer poly-T variants are associated with adverse outcomes: earlier age of onset in some clinical series; reduced grey-matter volume in Alzheimer-vulnerable regions and subtle cognitive decrements in cognitively normal carriers (the neuroimaging endophenotype evidence reviewed in Chapter VI); and, in longitudinal cohorts, differences in cognitive trajectory between Very-Long and Short homozygotes (Watts et al., 2019; Caselli et al., 2012). Because these comparisons are made within a single *APOE* genotype, they cannot be dismissed as ε4 effects in disguise, and they constitute the empirical core of the driver position.

A second strand of support is mechanistic rather than statistical, and the present dissertation gives it real weight: the expression and functional studies of Chapter V, which show that poly-T length modulates *TOMM40*/*APOE* transcription in reporter systems and human brain, supply a plausible biological pathway from genotype to phenotype. A genetic association is more credible when a mechanism is in hand, and for *TOMM40* a mechanism is at least partially in hand.

4.3 The Case Against

The sceptical literature is substantial and has grown more confident over time. Cruchaga and colleagues (2011), in association and expression analyses of *TOMM40* single-nucleotide polymorphisms, concluded that the *TOMM40* signal was not robustly independent of *APOE* and questioned whether poly-T length added predictive information beyond *APOE* genotype. Jun and colleagues (2012), in a comprehensive search for Alzheimer susceptibility loci across the *APOE* region using the large samples of the Alzheimer's Disease Genetics Consortium, found that the regional association was dominated by *APOE* and that apparent *TOMM40* effects were substantially attenuated or abolished after conditioning on *APOE* genotype. The broad thrust of the consortium-scale conditional analyses has been that, once *APOE* is properly accounted for, little independent *TOMM40* signal remains in case–control risk.

A further difficulty is the **inconsistency of the very-long allele's direction of effect**. The VL allele has been reported as protective in some studies and as

harmful in others, an instability that is difficult to reconcile with a single, simple causal mechanism and that may reflect differences in poly-T binning conventions, in the ancestral composition of samples, and in the *APOE* backgrounds against which VL was measured. Ancestry-aware analyses have underlined the point: the *TOMM40–APOE* linkage structure differs markedly between populations of European and African ancestry, and the poly-T association does not transfer cleanly across these backgrounds (the local-ancestry analyses of the late 2010s).

4.4 A Measured Verdict

The honest synthesis is that the question of independence remains formally unresolved at the level of human population genetics, and that the weight of the consortium-scale conditional evidence currently favours the view that *TOMM40* poly-T adds little to *APOE* for the prediction of case–control *risk*. This dissertation accepts that verdict for risk. It declines, however, to extend the verdict to two adjacent claims that the risk analyses do not actually address.

The first is the claim about **age of onset and trajectory** rather than lifetime risk. Roses' original signal was an onset-timing effect within ε3 backgrounds, and the consortium risk analyses — designed to detect case–control association — are not the ideal instrument for adjudicating a quantitative effect on the *timing* of disease among those who will develop it. The endophenotype evidence within ε3/ε3 (Chapter VI), which concerns timing and trajectory rather than dichotomous risk, is less easily dismissed.

The second is the claim about **mechanism**. Even if the poly-T tract carried *no* independent statistical signal — even if it were a pure passenger of *APOE* in every population — TOM40 the channel would remain a load-bearing node in the molecular cascade of Chapter II, and the abundance of the channel would remain a determinant of bioenergetic ceiling. The genetic-statistical question and the molecular-mechanistic question are not the same question, and a negative answer to the first does not entail a negative answer to the second. Much of the confusion in the *TOMM40* literature, in the author's view, comes from conflating them. Chapter VIII makes the separation explicit.

Chapter V — From Sequence to Expression

5.1 Why an Intronic Repeat Could Be Functional

A length polymorphism in an intron has no effect on protein sequence and is, on its face, an unpromising candidate for a functional variant. The hypothesis that the *TOMM40* poly-T tract nonetheless matters rests on the proposition that the tract is a *cis*-regulatory element — that its length influences the transcription, splicing, or message stability of the genes in its neighbourhood, namely *TOMM40* itself and the adjacent *APOE*. Homopolymeric tracts are known to influence local chromatin and transcription-factor occupancy, to affect nucleosome positioning, and to modulate the behaviour of nearby regulatory elements, so the proposition is biologically reasonable even if the specific mechanism remains incompletely defined.

5.2 The cis-Regulatory Evidence

The most direct support comes from the work of Chiba-Falek, Linnertz, and colleagues, who tested the regulatory hypothesis in reporter systems and in human brain tissue. In luciferase reporter assays, constructs bearing the Very-Long poly-T drove significantly higher expression than constructs bearing the Short poly-T, and the magnitude of the length effect was tissue-dependent, being greater in neuroblastoma-derived cells than in hepatoma-derived cells — consistent with a brain-relevant regulatory function (Linnertz et al., 2014). Analyses of allele-specific expression in human brain provided complementary evidence that poly-T length is associated with differential expression of *TOMM40* and *APOE* messages. Bekris and colleagues (2012) similarly implicated regional enhancers within the *APOE* locus in the coordinate regulation of both *TOMM40* and *APOE*, reinforcing the picture of a shared regulatory architecture spanning the two genes.

These results establish the key enabling premise of the driver hypothesis: that poly-T length is *capable* of modulating expression of the very genes whose products matter for the disease. They do not, by themselves, establish that this modulation is

what drives the clinical associations — reporter expression and brain mRNA are several inferential steps removed from age of onset — but they convert the genetic association from a bare statistical correlation into a candidate causal pathway with an identifiable first step.

5.3 TOMM40 RNA in the Alzheimer Brain

If poly-T length matters by setting *TOMM40* expression, then *TOMM40* expression should itself be perturbed in the disease — and the direction of that perturbation should, in principle, be interpretable. The literature here is genuinely difficult, for a technical reason that deserves emphasis: the human genome carries multiple *TOMM40* pseudogenes that produce highly homologous RNAs, so that naïve measurements of "*TOMM40* mRNA" are contaminated by pseudogene transcripts and the published direction of change has been inconsistent.

Lee and colleagues (2021) addressed this by developing an assay specific to the primary *TOMM40* transcript, and reported that *TOMM40* RNA is *up*-regulated in Alzheimer post-mortem brain, and that elevated *TOMM40* RNA is associated, in oxidatively stressed cells, with *decreases* in mitochondrial DNA copy number and mitochondrial membrane potential. The interpretation offered was that differential *TOMM40* transcription in the brain is a marker of, and possibly a contributor to, mitochondrial dysfunction. The apparent paradox — that higher *TOMM40* message accompanies worse mitochondrial outcomes — is most naturally read as a compensatory or stress-induced response, in which a failing import system upregulates the message for its central channel without restoring functional channel abundance (recall from Chapter I that channel abundance is the output of an assembly pipeline, not of transcription alone). The episode is a caution against assuming that "more *TOMM40* message" maps simply onto "more import capacity."

5.4 Suppression, Cholesterol, and Loss-of-Function Phenotypes

Complementary evidence comes from the opposite manipulation. Experimental suppression of *TOMM40* in neuronal systems has been reported to produce molecular and behavioural phenotypes reminiscent of Alzheimer's disease, including disturbances of neuronal cholesterol homeostasis — a finding that connects *TOMM40* loss-of-function to the lipid-metabolic disturbances that are central to *APOE* biology and to the mitochondria-associated-membrane pathology discussed in the companion bioenergetic thesis. That reducing TOM40 should perturb cholesterol handling is mechanistically coherent: mitochondria-associated ER membranes are a principal site of cholesterol and phospholipid metabolism, and mitochondrial dysfunction propagates readily into lipid dyshomeostasis. The loss-of-function evidence strengthens the case that TOM40 abundance is a phenotypically consequential variable, whatever the resolution of the poly-T statistics.

5.5 Reconciling Genotype, Expression, and Threshold

The pieces can now be assembled into the causal pathway that the driver hypothesis requires, with its uncertainties marked honestly. Poly-T length is *capable* of modulating *TOMM40*/*APOE* expression (§5.2, established in reporters and brain mRNA); *TOMM40* expression and TOM40 abundance are phenotypically consequential (§5.3–5.4, established in disease brain and loss-of-function models); and TOM40 abundance sets the import bottleneck that determines bioenergetic ceiling (Chapter I, established in import biology). The chain from inherited poly-T length to age of onset is therefore *mechanistically continuous* — each link is supported — even though the *magnitude* of the end-to-end effect, and its separability from *APOE*, remain contested at the population level. The driver hypothesis is, in other words, not biologically implausible; it is statistically unproven. That is a meaningfully different epistemic position from "refuted," and Chapter VIII will insist on the distinction.

Chapter VI — Endophenotypes and the Preclinical Window

6.1 The Logic of the Endophenotype

The dichotomous case–control phenotype — demented versus not — is a blunt instrument for a gene whose hypothesised effect is on *timing* and on the *slope* of decline. A more sensitive approach measures continuous, quantitative traits that lie on the causal path between genotype and clinical disease: regional brain volumes, memory performance, and molecular-imaging markers of pathology. Such endophenotypes can be measured in cognitively normal people years or decades before any clinical threshold is crossed, and — crucially for the *TOMM40* question — they can be measured *within* a single *APOE* genotype, so that the ε4 confound is removed by design. The endophenotype literature is therefore the natural home of any *APOE*-independent *TOMM40* effect, and it is where the driver hypothesis is at its strongest.

6.2 Grey Matter: Precuneus and Posterior Cingulate

Johnson and colleagues (2011) examined cognitively healthy, late-middle-aged *APOE* ε3/ε3 adults — the design that holds ε4 constant — and reported a dose-dependent relationship between Very-Long poly-T burden and reduced grey-matter volume: progressing from no VL allele, to S/VL heterozygotes, to VL/VL homozygotes, grey-matter volume declined in the ventral posterior cingulate and medial ventral precuneus, regions among the earliest affected in Alzheimer's disease. Honea and colleagues (2020) extended the finding in cognitively normal older ε3/ε3 adults, again linking longer poly-T variants to decreased regional grey matter. That a *TOMM40* length effect on the structure of Alzheimer-vulnerable cortex is detectable in people with no ε4 allele and no cognitive impairment is among the more persuasive pieces of evidence in the entire literature, precisely because the design forecloses the ε4 explanation.

6.3 Hippocampal Volume: An Honest Inconsistency

Candour requires noting that the structural findings are not uniform. Studies of hippocampal volume specifically — as distinct from precuneus and posterior cingulate — have been inconsistent, with some population-based samples of cognitively intact older adults finding no influence of either *APOE* or *TOMM40* on hippocampal volume or episodic memory. The discrepancy may reflect the region examined (the early *TOMM40* signal appears more reliably in posterior-medial cortex than in the hippocampus proper), the age and ascertainment of the sample, or the poly-T binning convention. The inconsistency is real and is reported here without minimisation; it is part of why the *TOMM40* endophenotype literature, though suggestive, has not closed the case.

6.4 Cognition and Longitudinal Trajectory

On the cognitive side, the relevant comparisons again hold *APOE* constant. Within ε3/ε3 carriers, VL/VL individuals have been reported to underperform S/S individuals on specific memory measures — notably primacy retrieval from verbal list learning, a pattern that mirrors early Alzheimer's disease — and longitudinal modelling of cognitive aging has detected *TOMM40* effects on the rate of decline (Caselli et al., 2012). Watts and colleagues (2019), working specifically within *APOE* ε3 homozygotes, examined the relationship of poly-T length to baseline and longitudinal cognition and contributed to the evidence that the locus modulates cognitive trajectory independent of *APOE* allele. These trajectory effects are the cognitive counterpart of the structural findings of §6.2, and they share the same methodological strength: they are measured where there is no ε4 to confound them.

6.5 Molecular Imaging

Molecular-imaging studies have begun to connect poly-T length to the pathological hallmarks themselves. Work using the FDDNP positron-emission-tomography ligand, which binds both amyloid and tau, reported associations between longer poly-T variants and higher medial-temporal ligand binding, suggesting that the genetic effect on structure and cognition is accompanied by an effect on the deposition of pathology. This evidence is younger and thinner than the structural and cognitive literatures, and the FDDNP ligand's dual amyloid/tau binding complicates interpretation, but it points in the same direction: a graded *TOMM40* effect detectable in the preclinical window.

6.6 The Endophenotype Verdict

Taken together, the endophenotype evidence supports a modest but real conclusion that the case–control risk literature of Chapter IV does not reach: *within* the ε3/ε3 background, longer *TOMM40* poly-T variants are associated with earlier and steeper structural, cognitive, and possibly pathological changes consistent with incipient Alzheimer's disease. This is the strongest available form of the driver hypothesis — strongest because it is measured where the ε4 confound cannot operate — and it is the empirical basis on which a *TOMM40*-containing risk algorithm was ultimately taken into a prospective prevention trial, the subject of Chapter VII.

Chapter VII — Translation: The TOMMORROW Trial

7.1 From Marker to Prospective Test

Every association reviewed so far is retrospective or observational. The decisive test of a risk marker is prospective: specify the algorithm in advance, enrol people on the basis of it, and ask whether the predicted events actually occur at the predicted rates. *TOMM40* is one of very few Alzheimer risk markers to have been subjected to such a test, in the TOMMORROW trial — an effort, led by Roses and colleagues and ultimately conducted by Zinfandel Pharmaceuticals with Takeda, that was simultaneously a biomarker-qualification study and a phase-3 prevention trial (Burns et al., 2019, 2021).

7.2 The Biomarker Risk-Assignment Algorithm

At the heart of TOMMORROW was a pre-specified **biomarker risk-assignment algorithm (BRAA)** that combined three inputs — *TOMM40* rs10524523 (poly-T) genotype, *APOE* genotype, and the participant's age — to classify cognitively normal older adults as high or low risk of developing mild cognitive impairment due to Alzheimer's disease within roughly five years. The algorithm is the concrete embodiment of the entire driver hypothesis: it asserts that poly-T length, combined with *APOE* and age, carries enough prognostic information to enrich a trial population for imminent converters. Qualifying that algorithm — having a regulator accept it as a validated prognostic biomarker — was the trial's first and arguably more important objective.

7.3 The Therapeutic Hypothesis: Low-Dose Pioglitazone

The trial's second objective was therapeutic. Participants classified as high-risk by the BRAA were randomised to low-dose **pioglitazone** — a thiazolidinedione PPAR-γ agonist used clinically as an insulin sensitiser, hypothesised to act in this setting through improvement of mitochondrial biogenesis and cerebral glucose metabolism — or to placebo; low-risk participants received placebo and served to anchor the algorithm's calibration. The mechanistic rationale is worth noting because it is congruent with this dissertation's frame: pioglitazone was chosen as a *bioenergetic* intervention, intended to raise the metabolic ceiling of at-risk neurons, in a trial that selected those neurons' owners with a *bioenergetic* gene. The hypothesis and the stratifier shared a theory.

7.4 Outcomes: A Double Result

TOMMORROW returned a result of two parts, and both parts matter.

On the **therapeutic** question, the trial was negative: low-dose pioglitazone did not delay the onset of mild cognitive impairment due to Alzheimer's disease relative to placebo among high-risk participants. The drug arm failed, and the study was halted early for futility on the efficacy endpoint.

On the **biomarker** question, the result was suggestive but inconclusive, and its inconclusiveness was a consequence of the therapeutic failure. The genetic algorithm did stratify risk in the predicted direction: among placebo recipients, the high-risk group experienced roughly three times the rate of progression events of the low-risk group — a substantial enrichment, and exactly what the BRAA was designed to produce. But because the trial was terminated early for the futility of the drug, it accrued fewer events than planned, and the algorithm's stratification, though in the right direction and of meaningful magnitude, did not reach the pre-specified threshold for formal biomarker qualification. The investigators were careful to state that, the study having stopped short of completion, the biomarker findings could only be considered exploratory.

7.5 Reading the TOMMORROW Result

What should be concluded from a trial in which the drug failed and the biomarker nearly succeeded? Three readings are defensible and they are not exclusive.

The first reading is **deflationary**: the algorithm did not meet its qualification threshold, the *TOMM40* component's contribution over *APOE* and age was never isolated within the BRAA, and a three-fold enrichment that misses significance is, formally, a null. On this reading TOMMORROW neither validated *TOMM40* nor refuted it; it simply ran out of events.

The second reading is **constructive**: a pre-specified genetic algorithm prospectively enriched a cognitively normal population three-fold for imminent progression — a non-trivial achievement for any Alzheimer prognostic, and a direction-correct prospective replication of the observational signal. That it missed significance for want of events is a feature of the early stop, not of the biology, and a completed trial might well have qualified it.

The third reading is **methodological**, and it is the one this dissertation emphasises: TOMMORROW is a model of how a mechanistically motivated genetic stratifier *should* be tested — specified in advance, embedded in a prospective design, anchored by a placebo low-risk arm — and its partial result is more informative than a shelf of retrospective associations precisely because it was prospective. The lesson for the field is not that *TOMM40* failed but that genetic-risk algorithms must be tested in trials large enough, and run long enough, to accrue the events on which their qualification depends.

7.6 Therapeutics at the Gate

Whatever the fate of the poly-T marker as a stratifier, the import-gate framework has therapeutic implications that the TOMMORROW design barely began to explore. The organising difficulty is the one established in Chapter II: the central lesion is a *physical channel arrest* — transmembrane-arrested APP plugging TOM40 — that no existing drug readily reverses. A rational strategy therefore does not wait for a single agent to "unblock the channel" but intervenes along the whole trajectory, from the supply of the plugging species to the matrix endpoint of what gets through. Five intervention points can be ordered from the most mechanism-specific to the most downstream, each marked here for its current level of evidence.

7.6.1 Reducing the Substrate: Lowering APP and Its Mistargeting

Because the arrest scales with APP load (§2.1), lowering the cytosolic, non-glycosylated APP pool that is mistargeted to the mitochondrion reduces the supply of the plugging species at its source. Candidate approaches include *APP*-lowering antisense oligonucleotides, enhancement of non-amyloidogenic α-secretase (ADAM10) processing, and β-secretase modulation. The caveat is substantial: this is the conventional amyloid axis, and BACE1 inhibitors failed in clinical trials with cognitive worsening, while the plaque-clearing antibodies (lecanemab, donanemab) act on the *extracellular* compartment and do not obviously address the *intracellular* APP that jams the gate. The import-gate framing nonetheless reframes the target as intraneuronal APP rather than fibrillar plaque. *Evidence: indirect for the import endpoint specifically*.

7.6.2 Clearing the Clog: Augmenting Import-Stress Quality Control

The cell already possesses machinery dedicated to precisely this lesion — stalled precursors jamming the translocase. The mitochondrial AAA-ATPase ATAD1 (the mammalian Msp1) extracts mistargeted membrane proteins; p97/VCP-dependent retrotranslocation and outer-membrane ubiquitin ligases such as MARCH5 remove stalled precursors to the proteasome; the protease OMA1 eliminates arrested import intermediates upon depolarisation; and the mitochondrial compromised-protein-import response (mitoCPR), characterised in yeast by Weidberg and Amon (2018), clears clogged channels under import stress. Pharmacological augmentation of this clog-clearance machinery is, of all the strategies here, the one that maps most directly onto the lesion — it aims to pull the arrested APP out of TOM40 rather than to compensate around it. *Evidence: preclinical and conceptual; no clinical agent yet, but the mechanism-matched frontier.*

7.6.3 Clear and Rebuild: Mitophagy and Biogenesis

A channel that cannot be unplugged can be destroyed along with its organelle and replaced. This strategy also corrects the PINK1 miscalibration that obstruction itself induces (§2.5). Mitophagy inducers — urolithin A, NAD□ precursors (nicotinamide riboside, NMN) acting through the sirtuin–PGC-1α axis, spermidine, and actinonin — were shown by Fang and colleagues (2019) to reduce amyloid-β and tau pathology and reverse cognitive deficits in Alzheimer models; urolithin A and nicotinamide riboside have advanced to human trials. Coupled mitochondrial *biogenesis* (PGC-1α activation) rebuilds the pool with fresh, unobstructed TOM complexes — the same biogenetic logic that motivated the pioglitazone arm of TOMMORROW, which failed on cognition but whose rationale survives the trial. *Evidence: strong preclinical; early clinical.*

7.6.4 Breaking the Vicious Cycle: Sustaining the Membrane Potential

The arrest lowers the membrane potential, and because the TIM23 step is potential-dependent, the falling potential further degrades import — a self-deepening loop (§1.5). Propping up the potential keeps residual import running. Alternative electron carriers (methylene blue, which feeds Complex IV — the very complex the lesion starves), ketone bodies and succinate (bypassing upstream electron-transport deficits), and the cardiolipin-stabilising peptide elamipretide (SS-31, which supports cristae and Complex IV) all act to sustain bioenergetic competence in the face of partial obstruction. *Evidence: mixed — methylene blue equivocal in AD trials; elamipretide preclinically strong and in trials for primary mitochondrial disease.*

7.6.5 Neutralising the Matrix Endpoint: Aβ–ABAD Disruption

What transits the gate must still be defused inside. The Aβ–ABAD complex (§2.2) is a uniquely druggable matrix endpoint because small molecules reach the matrix where antibodies cannot: the ABAD-derived decoy peptide (ABAD-DP), the covalent probe AG18051, and the frentizole-derivative class disrupt the complex while sparing ABAD's essential mitochondrial-RNase-P scaffold function, and mito-targeted antioxidants (MitoQ) blunt the reactive-oxygen output the complex generates. This arm is developed in full in the companion ONS pharmacology monograph on AG18051. *Evidence: preclinical, but a distinctively accessible target.*

7.6.6 The Integrative Logic and Genotype Stratification

No single agent unblocks TOM40 today, and because the channel is *both obstructed and load-bearing* the defensible near-term play is combination: reduce the APP substrate (7.6.1), restore mitophagy and biogenesis to clear and replace the un-repairable organelles (7.6.3), sustain the membrane potential to halt the self-deepening cycle (7.6.4), and disrupt the ABAD endpoint (7.6.5) — with clog-clearance enhancement (7.6.2) as the mechanism-specific frontier to develop. The broader logic of genotype-stratified prevention, meanwhile, survives TOMMORROW intact: stratifying trial populations by inherited bioenergetic capacity — whether by *APOE*, *TOMM40* poly-T, mitochondrial DNA haplogroup, or FDG-PET hypometabolism — remains a sound way to enrich for the patients in whom propping up the gate should matter most, namely those whose inherited import reserve is lowest. That the specific TOMMORROW realisation did not complete is a fact about one trial, not about the strategy.

Chapter VIII — Synthesis: The Two-Faced Gene

8.1 Separating Two Questions That the Literature Conflates

The single most important analytical move this dissertation can make is to insist that two questions about *TOMM40* are distinct and have different answers:

- **The statistical question.** Does the *TOMM40* poly-T tract carry information about Alzheimer's disease risk that is independent of the *APOE* allele with which it is in linkage disequilibrium? *Provisional answer: for dichotomous case–control risk, largely no, on current consortium-scale evidence; for age of onset and preclinical trajectory within ε3 backgrounds, plausibly yes, on current endophenotype evidence.*
- **The mechanistic question.** Is TOM40, the channel that *TOMM40* encodes, a causally important node in the pathogenesis of Alzheimer's disease? *Answer: yes, with high confidence, independent of the statistical question.*

These two questions have been chronically conflated, with the result that negative findings on the first have been read as though they bore on the second. They do not. A gene can be a near-perfect statistical passenger of its neighbour and still encode a protein that is mechanistically load-bearing. *TOMM40* is, on present evidence, plausibly exactly that.

8.2 Where TOM40 Sits in the Cascade

Re-read through the synthesis, the molecular chapters and the genetic chapters describe the same node from two directions. From the molecular direction (Chapter II), TOM40 is the chokepoint that amyloid-β obstructs and depletes, closing the feedback loop in which proteinopathy throttles the import gate whose failure helped generate the proteinopathy. From the genetic direction (Chapters III–VII), *TOMM40* is a candidate inherited modifier of the abundance of that same chokepoint, and hence of the bioenergetic ceiling whose height the Mitochondrial Cascade Hypothesis makes decisive for onset timing. The channel is simultaneously a *victim* of the cascade and a *parameter* of it — obstructed by the disease while helping set the threshold at which the disease ignites. The two readings are not in tension; the closed loop of §2.5 requires both.

8.3 The Chimera: A 2024 Twist That May Dissolve the Confound

The most provocative recent development bears directly on the central confound. In 2024 a transcriptional phenomenon was reported in which *TOMM40* read-through transcription into the adjacent *APOE* gene generates a spliced *TOMM40–APOE* messenger RNA chimera — termed T9A2 — detectable in human neurons and other tissues. Translation of this chimera tethers apoE to a near-full-length TOM40 that is targeted to mitochondria. Strikingly, the *APOE3* version of the chimera was reported to boost mitochondrial bioenergetic capacity and reduce oxidative stress substantially more than the *APOE4* version, supplying an isoform-specific, mitochondria-localised mechanism that links the two highest-risk Alzheimer genes at the level of a single fused gene product.

If this finding stands, its implications for the present thesis are considerable. It would mean that *TOMM40* and *APOE* are not merely confounded *statistically* by linkage disequilibrium but coupled *mechanistically* at the mitochondrion — that the apoE isoform and the TOM40 channel are, in this chimeric product, literally the same molecule, acting together at the organelle whose import gate is the subject of this work. The fifteen-year struggle to assign signal to one gene or the other might then rest on a false premise: at the level of mechanism, in at least this pathway, the two genes do not have separable effects to assign. The confound that has frustrated

the genetics could turn out to be a clue to the biology. This is, at present, a single-study finding awaiting replication, and it is flagged as such; but it is precisely the kind of result toward which the convergence logic of this dissertation has been pointing.

8.4 What the ONS Methodology Adds

The Organic Network Synthesis methodology under which this work is prepared is built to surface exactly the structure that *TOMM40* exhibits: a node onto which multiple upstream pathologies converge. Amyloid-β converges on the gate from one side (Chapter II); α-synuclein converges on the partner receptor in a parallel disease; apoE4 fragments converge on the mitochondrial surface and import apparatus; and the inherited poly-T tract may tune the gate's abundance from birth. The methodological yield of placing TOM40 at the centre of its own monograph — rather than treating it as a single subsection of a bioenergetic thesis — is that the convergence becomes visible as a structure in its own right. The gate is not one mechanism among many; it is where several mechanisms meet.

Chapter IX — Falsifiable Predictions and Open Questions

9.1 Predictions

The framework advanced here generates predictions that are, in principle, falsifiable; they are offered in the spirit of the ONS methodology's insistence that a synthesis earn its keep by exposing itself to refutation.

Prediction 1 — Import capacity tracks poly-T within ε3/ε3. In *APOE* ε3/ε3 neurons (including iPSC-derived neurons stratified by poly-T length), mitochondrial protein-import flux and TOM40 channel abundance will be measurably lower in Very-Long than in Short carriers, with a graded relationship across genotypes. A failure to detect any difference in import capacity between poly-T classes within ε3/ε3 would falsify the cis-regulatory-to-import limb of the driver hypothesis.

Prediction 2 — Onset-timing signal survives where risk signal does not. Quantitative analyses of age of onset and of preclinical decline rate within ε3/ε3 cohorts will retain a poly-T effect even in samples and meta-analyses in which dichotomous case–control risk shows no independent *TOMM40* signal after conditioning on *APOE*. The dissociation between a null risk effect and a non-null timing effect is the empirical signature of the position taken in §4.4.

Prediction 3 — TOM40 obstruction precedes plaque. In longitudinal animal and human-tissue studies, biochemical evidence of TOM40 channel obstruction and reduced TOM40 abundance will be detectable before extracellular plaque deposition in vulnerable neuronal populations, consistent with the cascade placing import failure upstream of, not downstream of, frank amyloid pathology.

Prediction 4 — PINK1 mishandling under obstruction. Acute pharmacological or amyloid-β-mediated obstruction of TOM40 will stabilise PINK1 on the outer membrane of mitochondria whose membrane potential is preserved, demonstrating the miscalibration of mitophagy by import blockade independent of depolarisation (§2.5).

Prediction 5 — The chimera is isoform-graded in human neurons. The T9A2 *TOMM40–APOE* chimera will be detectable in human neurons at levels and with bioenergetic consequences that differ by *APOE* isoform, with the ε3 chimera conferring greater mitochondrial benefit than the ε4 chimera; and manipulation of read-through transcription will modulate mitochondrial function in an isoform-dependent manner (testing §8.3).

Prediction 6 — Bioenergetic enrichment beats the BRAA's drug. A genotype-stratified prevention trial that pairs a *TOMM40*/*APOE*/age enrichment strategy with a mitophagy-restoring or NAD□-repleting intervention (rather than pioglitazone), powered to completion, will show both a qualifiable risk algorithm and a non-null therapeutic effect — testing whether TOMMORROW's biomarker result was limited by its drug rather than its stratifier.

Prediction 7 — Import support is broadly protective. Interventions that augment mitochondrial import capacity or relieve channel obstruction will reduce pathology across more than one proteinopathy model (Alzheimer and Parkinson), consistent with the gate being a convergent node rather than an Alzheimer-specific one (§2.3).

Prediction 8 — Clog-clearance relieves the obstruction. Pharmacological or genetic enhancement of the mitochondrial import-stress clearance machinery (ATAD1/Msp1, p97/VCP-dependent extraction, OMA1, or the mitoCPR-type response) will reduce the burden of transmembrane-arrested APP at TOM40, restore the import of nuclear-encoded Complex IV subunits, and improve respiration in APP-overexpressing models — and will do so without requiring any reduction in extracellular amyloid. A failure of clog-clearance enhancement to relieve import obstruction would indicate that the arrested-APP complexes are not accessible substrates for the quality-control machinery, and would weaken the §7.6.2 therapeutic limb.

9.2 Open Questions

- Does the poly-T tract act primarily on *TOMM40* expression, on *APOE* expression, or on both coordinately, and is the relevant tissue neuronal, glial, or both?

• Why is the direction of the Very-Long allele's effect unstable across studies, and is the instability fully explained by binning conventions and ancestry, or does it reflect a genuine context-dependence of the variant's effect?

• Is the up-regulation of *TOMM40* RNA in Alzheimer brain compensatory, contributory, or epiphenomenal, and does it translate into any change in functional channel abundance given the assembly-pipeline bottleneck of §1.4?

• Does the T9A2 chimera replicate, and if so what fraction of the *APOE*/*TOMM40* mitochondrial phenotype does it account for relative to secreted apoE and apoE fragments?

• Can a prospective trial ever be powered to qualify a *TOMM40*-containing algorithm, given the event rates and durations involved, and is the marginal information of poly-T over *APOE* and age large enough to be worth the genotyping?

Chapter X — Conclusion

This dissertation set out to take a single gene — one whose name appears in the literature as both a footnote to *APOE* and a candidate Alzheimer gene in its own right — and to give it the sustained, integrated treatment that its position warrants. The case for doing so rests on a fact of biology that the genetics has tended to obscure: *TOMM40* encodes the central channel of the mitochondrial import gate, the narrow pore through which the entire nuclear-encoded mitochondrial proteome must pass, and that gate is a chokepoint controlling replacement, surveillance, and disposal in the most metabolically extravagant cells of the body.

The molecular evidence for the channel's importance is strong and, in the author's judgement, not seriously in doubt: amyloid-β obstructs and depletes TOM40 from the cytosolic face while traversing it to poison Complex IV from within; α-synuclein inflicts the parallel lesion at TOM20 in Parkinson's disease; apoE4 fragments target the mitochondrial surface and its import apparatus; and obstruction of the gate miscalibrates the PINK1 sensor, closing a feedback loop in which proteinopathy throttles the import machinery whose failure helped generate it. The genetic evidence is genuinely contested: the poly-T tract's independence from *APOE* for case–control risk is doubtful on consortium-scale conditional analysis, while its association with age of onset and preclinical trajectory within ε3/ε3 backgrounds — measured precisely where the ε4 confound cannot reach — is more robust; and the one prospective test, TOMMORROW, returned a negative drug result and a directionally-correct but underpowered biomarker result that neither validated nor refuted the marker.

The synthesis this dissertation offers is to refuse the conflation that has dogged the field. Whether or not the poly-T tract earns its place in a risk algorithm is a statistical question about a genetic marker; whether TOM40 is a load-bearing node in the disease is a mechanistic question about a protein; and the answers can, and on present evidence do, diverge — doubtful for the first, confident for the second. The 2024 discovery of a *TOMM40–APOE* chimera that fuses the two genes' products at the mitochondrion raises the possibility that the divergence is itself an artefact of asking the genetics to apportion an effect that the biology does not partition — that at the organelle, the channel and the lipoprotein are doing one thing together. If that is so, the long argument over which neighbour owns the signal will have been, in the

deepest sense, the wrong question. The right question was always what happens at the gate.

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A 2024 preprint (bioRxiv 2024.10.09.617477; indexed at PubMed 39416128) reporting the TOMM40–APOE *read-through chimera (T9A2) and its isoform-specific mitochondrial effects is cited in Chapter VIII as an unreplicated primary finding; details are given in text pending peer-reviewed publication.*