Mapping the Vascular Dimension onto the Genetic Dimension

A Phase-Resolved Synthesis of the Cerebrovascular Genome as Phase 0 of Late-Onset Neurodegeneration

Tight Junctions, Pericytes, Transcytosis, the VCAM-1 Gateway, APOE Re-Read as Vascular, the Mendelian Small-Vessel-Disease Core, and the Glymphatic Genome — Mapped onto the Three Temporal Phases of the Collapse Architecture

ONS Methodology — First Principles — Doctoral Thesis

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“The cerebrovasculature is not a conduit. It is the first cellular substrate at which the systemic aging program imposes a load on the central nervous system — and it is the first substrate at which the inherited architecture of late-onset neurodegeneration begins to load-bear.”

— ONS Methodology Working Notes, 2026

For Berislav Zlokovic and Axel Montagne, whose dynamic-contrast-enhanced imaging program supplied the in vivo evidence that the blood-brain barrier fails before the parenchyma does; for Tony Wyss-Coray and Hanadie Yousef, whose identification of the VCAM-1 gateway gave the vascular dimension its molecular center; and for Oskar Fischer, whose first description of the senile plaque in 1907 noted, in passing, the vascular relations that twelve decades of parenchymal-centric reading would later set aside.

ONS METHODOLOGY — FIRST PRINCIPLES

Companion to the Collapse Trilogy and the First-Principles Volumes

I. Convergent Synaptic Collapse

II. Homeostatic Microglial Collapse

III. Bioenergetic Collapse

IV. The Tryptophan Partition

V. The Genetic Architecture of Neurodegeneration

VI. Mapping the Vascular Dimension onto the Genetic Dimension □ this volume

The Collapse trilogy and the Genetic Architecture volume have read late-onset neurodegeneration through three sequential parenchymal phases — a Phase I bioenergetic substrate in the locus coeruleus, a Phase II microglial substrate in the hippocampus, and a Phase III matrix substrate at the cortical perineuronal net. The present volume adds a fourth layer, prior to the three, and argues that the cerebrovasculature is itself a genetically structured cellular substrate whose load-bearing trajectory across the third through fifth decades constitutes a *Phase 0* — the substrate at which inherited risk first becomes load-bearing and through which every subsequent phase is conditioned.

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Abstract

The genetic architecture of late-onset neurodegeneration, as it has been read by three decades of Mendelian sequencing, GWAS meta-analyses, and single-cell expression atlases, has located its load-bearing biology in the parenchyma. APP and PSEN1/2 are read as neuronal genes; MAPT and GRN as neuronal-glial; TREM2 and the CD33/MS4A cluster as microglial; APOE as a lipid carrier expressed principally in astrocytes and microglia. The Collapse trilogy and its companion volume on the Genetic Architecture of Neurodegeneration extended this parenchymal reading by ordering the genes into three sequential temporal phases — a Phase I anchored to the locus coeruleus and the NAD□-PARP-mitochondrial axis, a Phase II anchored to hippocampal microglia and oligodendrocyte ferroptosis, and a Phase III anchored to cortical perineuronal nets and the MMP-9-driven matrix-collapse program. Across these three phases the cerebrovasculature has been treated as either a passive conduit or a late-stage consequence of parenchymal failure. This treatment is not sustainable in 2026. The cerebrovasculature is itself a genetically structured cellular system whose inherited variation is load-bearing for late-onset neurodegeneration on a trajectory that begins before the bioenergetic ignition of the locus coeruleus and conditions every subsequent phase of the disease.

This dissertation advances the thesis that the vascular dimension has a genetic dimension, and that the genes implicated in cerebrovascular function — at the tight junction, the pericyte-endothelial interface, the transporter geometry, the transcytotic suppression machinery, the VCAM-1/BACE2 axis, the basement membrane, and the glymphatic clearance pathway — together constitute a *Phase 0* architecture in the phase-resolved framework of the Collapse program. The work is organized in ten analytical chapters. Chapter I develops the Phase 0 framework as a temporal-architectural extension of the three-phase Collapse model. Chapter II treats the tight-junction gene complex (CLDN5, OCLN, TJP1, TJP2, JAM-A/B/C, MARVELD2) as the molecular substrate of paracellular selectivity, with attention to the age-dependent reduction in CLDN5 expression as the proximate molecular event of blood-brain-barrier compromise. Chapter III treats the pericyte-endothelial axis

(PDGFB, PDGFRB, NOTCH3, HTRA1, FOXF2, FOXC1) as the cellular substrate of vascular stability, with the Mendelian small-vessel-disease genes NOTCH3 and HTRA1 as the high-penetrance anchors of the axis. Chapter IV treats the transcytosis and transporter geometry (MFSD2A, SLC2A1, LRP1, AGER, ABCB1, ABCG2, INSR) as the gene set that defines the curated interface across which the brain reads its systemic environment. Chapter V develops the VCAM-1 / BACE2 / α4β1 axis (VCAM1, BACE2, ADAM17, ITGA4, ITGB1, ICAM1) as the integrative vascular gateway through which the aged systemic milieu is transduced into a parenchymal signal, with explicit attention to BACE2 as the principal physiological sheddase of VCAM-1 and the gene whose pharmacological status under BACE-inhibitor therapy determines the vascular cost of amyloid-directed intervention. Chapter VI re-reads APOE as a vascular master variable, situating the cyclophilin-A / MMP-9 / claudin-5 pathway (PPIA, MMP9, TIMP3, LRP1) as the proximate molecular substrate of APOE4-driven blood-brain-barrier breakdown that the Montagne–Zlokovic program has documented in vivo. Chapter VII treats the Mendelian cerebral small-vessel-disease genes — NOTCH3 (CADASIL), HTRA1 (CARASIL), COL4A1 and COL4A2 (HANAC and porencephaly), TREX1 (RVCL-S), CTSA (CARASAL), and GLA (Fabry disease) — as the high-penetrance core of the vascular genome whose effector pathways anticipate, at the rare end of the allelic spectrum, the same vascular biology that the common-variant architecture modulates at small effect sizes. Chapter VIII treats the glymphatic clearance architecture (AQP4, GFAP, DTNA, DTNB, MLC1, FOXC1, PROX1) as the genetically encoded cerebrospinal-to-interstitial exchange system whose failure conditions the Phase II microglial substrate. Chapter IX maps the entire vascular genome onto the three-phase architecture of the Genetic Architecture dissertation, deriving the formal claim that Phase 0 is genetically encoded principally in the vascular Mendelian and SVD-associated genes and that each subsequent phase carries a vascular gene set whose action is mediated through the cerebrovascular substrate. Chapter X develops the therapeutic implications and falsifiable predictions, including the case for genotype-stratified anti-amyloid therapy (APOE × CAA × ARIA), pericyte-protective intervention in NOTCH3 and HTRA1 carriers, and the vascular biomarker (sVCAM-1, sPDGFRβ, CSF claudin-5) stratification of the Collapse-program clinical-trial design.

The dissertation concludes that the canonical Phase I/II/III architecture of late-onset neurodegeneration is incomplete without a Phase 0 vascular layer, that the genes that load-bear Phase 0 are largely orthogonal to the Phase I–III genome but

converge on it through three integrative nodes (APOE, TREM2, and the matrix-metalloproteinase family), and that the failure to model the vascular dimension as a genetically structured first stage is the principal explanation for the disappointing performance of stratification-naïve trial designs and the disproportionate ARIA toxicity of anti-amyloid monoclonal antibody therapy in APOE4 homozygotes. The vascular dimension is not a comorbidity. It is a phase, and it has a genome.

Keywords: cerebrovascular genetics, CADASIL, NOTCH3, HTRA1, COL4A1, claudin-5, MFSD2A, VCAM-1, BACE2, APOE, pericyte, blood-brain barrier, glymphatic clearance, AQP4, ARIA, temporal pharmacology, ONS Methodology, Collapse trilogy

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1. Introduction

1.1 The Research Problem

The genetic architecture of late-onset neurodegeneration has, over three decades of work, settled on a set of conventions whose collective effect is to locate the disease's load-bearing biology in the parenchyma. The Mendelian core — *APP*, *PSEN1*, *PSEN2*, *MAPT*, *GRN*, *SNCA*, *LRRK2*, *GBA*, *C9ORF72*, *TARDBP*, *FUS*, *SOD1*, *HTT* — is read in neuronal and glial terms; the common-variant architecture, anchored in single-cell expression atlases that consistently report microglial enrichment of the GWAS catalog, is read in microglial and lipid-handling terms; and the integrative master variable, *APOE*, is read as an astrocyte-and-microglia-expressed apolipoprotein whose cerebral function is principally the inter-cellular distribution of cholesterol within the parenchymal compartment. The companion volume on the Genetic Architecture of Neurodegeneration extended this reading by mapping each gene to a temporal phase: a Phase I anchored to the locus coeruleus and the NAD□ axis, a Phase II anchored to hippocampal microglia and oligodendrocyte ferroptosis, and a Phase III anchored to cortical perineuronal nets and the MMP-9-driven matrix collapse. The architecture is satisfying because it integrates Mendelian rarity with polygenic distribution, and it is satisfying because it specifies, at each phase, what cell types are load-bearing and through which effector pathways.

What the architecture does not specify is the vasculature. The cerebrovascular cellular compartment — brain endothelial cells, pericytes, vascular smooth muscle, perivascular macrophages, and the basement membrane that anchors the astrocytic endfoot — is treated, in the conventional reading, either as a passive conduit (a plumbing system whose only role is to deliver glucose and oxygen and remove waste) or as a late-stage downstream casualty of parenchymal pathology (the capillary degeneration secondary to amyloid deposition, the blood-brain-barrier compromise as a consequence of inflammation, the microbleeds as the iatrogenic cost of antibody therapy). On either reading, the vasculature has no genome of its own that bears on disease initiation. The Phase I/II/III architecture is read as parenchymal end-to-end.

This treatment cannot survive a careful reading of three convergent literatures. The first is the Zlokovic–Montagne program at the University of Southern California, which has established by dynamic contrast-enhanced MRI that blood-brain-barrier breakdown is detectable in the human hippocampus and medial temporal cortex in

the sixth decade — earlier than amyloid PET positivity, earlier than CSF tau elevation, earlier than the entry into the Phase II microglial substrate. The second is the Wyss-Coray–Yousef program at Stanford, which has identified soluble vascular cell adhesion molecule-1 (sVCAM-1) as the single most age-upregulated soluble protein in mammalian plasma and has demonstrated that brain endothelial VCAM-1 is the necessary mediator through which the aged systemic milieu impairs hippocampal neurogenesis and cognitive performance in aged mice. The third is the Mendelian small-vessel-disease literature, which has identified high-penetrance pathogenic variants in *NOTCH3* (CADASIL), *HTRA1* (CARASIL), *COL4A1* and *COL4A2* (HANAC and porencephaly syndromes), *TREX1* (retinal vasculopathy with cerebral leukoencephalopathy), *CTSA* (CARASAL), *GLA* (Fabry disease), and *FOXC1* (anterior-segment dysgenesis with cerebrovascular involvement) — a gene set that produces, at the rare end of the allelic spectrum, the same vascular phenotype that the common-variant architecture produces at small effect sizes and that the Wyss-Coray and Zlokovic programs have linked to the parenchymal trajectory of Alzheimer's disease.

The convergence of these three literatures forces the reading that the cerebrovasculature has a genetic architecture of its own, that this architecture is load-bearing for late-onset neurodegeneration, and that it acts at a temporal stage *prior* to the Phase I bioenergetic ignition of the locus coeruleus. The present dissertation calls this stage Phase 0 and devotes itself to the mapping of the vascular genome onto the genetic architecture of the Collapse program.

This dissertation addresses the question: **How does the vascular dimension of late-onset neurodegeneration map onto the genetic dimension, and what genes load-bear the phase that precedes the parenchymal trajectory?** The thesis advanced is that the vascular dimension is a Phase 0 architecture, that the genes load-bearing Phase 0 are largely orthogonal to the Phase I–III genome but converge on it through three integrative nodes — *APOE*, *TREM2*, and the matrix-metalloproteinase family — and that the failure to model Phase 0 as a genetically structured first stage is the principal explanation both for the disappointing performance of stratification-naïve trial designs and for the disproportionate ARIA toxicity of anti-amyloid monoclonal antibody therapy in APOE4 homozygotes.

1.2 Significance

The significance of the phase-zero reading is fivefold. First, it supplies a genetic interpretation of the temporal-precedence finding from the Zlokovic–Montagne program. If blood-brain-barrier breakdown precedes amyloid PET positivity, the precedence has a genetic substrate — the variants in *PDGFRB*, *NOTCH3*, *HTRA1*, *CLDN5*, *MFSD2A*, *COL4A1/2*, *AGER*, and the regulatory architecture of the *APOE-PPIA-MMP9-CLDN5* axis — and the substrate is itself heritable, modifiable, and (in principle) drug-targetable. The reading converts a phenomenological precedence into a genetic precedence and supplies the targets that intervention at Phase 0 would require.

Second, the phase-zero reading reorganizes the clinical-trial landscape. A trial of anti-amyloid monoclonal antibody therapy that enrolls participants whose Phase 0 substrate has already substantially failed — an APOE4 homozygote with detectable hippocampal BBB permeability on DCE-MRI, with elevated CSF sVCAM-1, with sPDGFRβ above the Montagne reference range — will dose into a vascular substrate that cannot tolerate the amyloid-clearance event, and the trial's ARIA toxicity will be disproportionate. The same trial enrolled with APOE3/3 participants whose Phase 0 substrate is intact would be expected to tolerate the same dose with substantially less ARIA. The phase-zero reading therefore makes a clear prediction about stratification: ARIA risk is a function of the Phase 0 vascular genome, and trial design must condition on the vascular substrate rather than on the parenchymal biomarker alone.

Third, the phase-zero reading clarifies the meaning of the *APOE* effect. *APOE* has the largest per-allele effect of any gene in the AD common-variant architecture, and the conventional reading attributes this effect to lipid distribution in the astrocytic-microglial compartment. The phase-zero reading attributes a substantial fraction of the *APOE4* effect to its vascular phenotype — its failure to suppress the *PPIA-MMP9* axis in pericytes, its compromise of claudin-5 at the brain endothelium, and its acceleration of the entry into the VCAM-1-gated vascular-microglial transition — and supplies a unified mechanism for the long-observed temporal precedence of APOE4-driven BBB breakdown over APOE4-driven amyloid accumulation. The vascular phenotype is the *first* phenotype of *APOE4* in human cohorts; the parenchymal phenotype is the second. The conventional reading has had the order backwards.

Fourth, the phase-zero reading supplies the integrative framework in which the Mendelian small-vessel-disease genes and the common-variant AD genes can be read together. CADASIL produces, in adult-onset autosomal-dominant fashion, the same imaging phenotype — confluent subcortical white-matter hyperintensities, microbleeds, lacunar infarcts — that the cerebral small-vessel-disease component of late-onset AD produces in autosomal-recessive-multifactorial fashion in the seventh and eighth decades. The CADASIL pathology terminates in vascular dementia in the sixth decade; the late-onset SVD pathology contributes to the cognitive impairment of mixed-pathology dementia in the eighth and ninth decades. The two trajectories run through the same vascular substrate at different penetrances and different ages, and the gene sets that drive them — *NOTCH3* and *HTRA1* in the Mendelian case, the *FOXF2-FOXC1* / *PITX2* / *EFEMP1* common-variant SVD loci and the *APOE* / *PICALM* / *BIN1* / *CR1* AD loci in the polygenic case — are best read as a single phase-zero architecture with high-penetrance and low-penetrance branches.

Fifth, the phase-zero reading supplies a unifying genetic substrate for a set of otherwise disparate clinical observations: the strong association of midlife hypertension with late-life dementia risk (which the framework attributes to vascular-substrate damage accruing across the third through sixth decades and entering the disease cascade through the same pericyte-endothelial axis that the Mendelian small-vessel-disease genes target); the protection conferred by lifelong physical activity (which the framework attributes to preservation of pericyte function and endothelial flow-mediated dilation); the strong inverse association of GLP-1-receptor agonist use with dementia incidence (which the framework attributes to vascular endothelial GLP-1 signaling and the preservation of MFSD2A-mediated DHA-LPC transport); and the high cardiovascular comorbidity of Alzheimer's disease at autopsy (which the framework attributes to shared vascular substrate failure rather than to coincidence).

1.3 Scope and Limitations

This dissertation is a synthetic review and integrative argument, not a report of original experimental or bioinformatic data. Its contribution is the integration of four distinct genomic literatures — the Mendelian-genetics literature on cerebral small-vessel disease, the GWAS literature on cerebrovascular and stroke phenotypes, the cell-type-resolved single-cell-transcriptomics literature on brain endothelial and pericyte expression, and the imaging-genetics literature on white-matter hyperintensities and BBB permeability — into a single analytical schema centered on the vascular dimension as Phase 0 of late-onset neurodegeneration. The work draws principally on Alzheimer's disease because the temporal-phase framework was developed for AD, but it engages comparably with vascular cognitive impairment, mixed dementia, and the broader cerebrovascular contribution to Parkinson's disease, frontotemporal dementia, and amyotrophic lateral sclerosis where the vascular evidence is informative.

The dissertation does not attempt to resolve whether all late-onset neurodegeneration begins as a vascular event, nor whether the Phase 0 substrate is the *causally upstream* variable in every patient. The phase-zero reading is consistent with multiple causal architectures — Phase 0 may initiate the cascade in some patients, may run in parallel with Phase I in others, and may be largely uninvolved in some monogenic forms (e.g., early-onset APP/PSEN-driven autosomal-dominant AD) where the Mendelian variant drives a parenchymal trajectory that may outrun any vascular contribution. What the framework asserts is that in the population that bears the bulk of the late-onset disease burden — the typical 65-to-90-year-old patient with sporadic AD, with comorbid vascular pathology, with cerebral microbleeds on MRI, and with the long midlife-vascular-risk-factor history that the epidemiology has consistently identified — Phase 0 is load-bearing and is genetically structured, and the trial-design implications of this finding are direct.

The dissertation also does not attempt to be exhaustive across all vascular genes. The vascular genome implicates several hundred loci across stroke, hypertension, atherosclerosis, and small-vessel-disease GWAS catalogs, and only a subset of these has been mapped to a clearly defined cerebrovascular phenotype with neurodegeneration relevance. The dissertation treats at length the genes whose effector function is best characterized; the broader catalog is touched on in context.

2. Literature Review and Methodology

2.1 The Vascular Hypothesis: Roy, Sherrington, Lassen, Iadecola

The vascular hypothesis of brain function predates the molecular genetics of neurodegeneration by more than a century. Roy and Sherrington's 1890 paper "On the regulation of the blood-supply of the brain" supplied the first quantitative demonstration that regional cerebral blood flow tracks regional metabolic demand and that the vasculature is, on this account, an active responsive system rather than a passive conduit. Niels Lassen's program in the 1950s and 1960s established the quantitative cerebral blood flow measurement by 133-xenon clearance and supplied the first regional maps of CBF in normal and diseased brains, including the now-classical demonstration of temporoparietal hypoperfusion in early Alzheimer's disease. Costantino Iadecola's program at Cornell, continuing through the 2000s and 2010s, has extended this work to the cellular biology of neurovascular coupling and has established the failure of the coupling system as one of the earliest detectable functional changes in the aged and the diseased brain. The vascular hypothesis of Alzheimer's disease, in its mature form, holds that vascular dysfunction is one of the earliest detectable substrates of the disease trajectory and that the parenchymal events of amyloid and tau accumulation are, in part, downstream of the vascular events.

2.2 The Mendelian Cerebral Small-Vessel-Disease Era (1993–2026)

The Mendelian genetics of cerebral small-vessel disease began with the 1993 mapping of CADASIL (cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy) to chromosome 19q12 and the 1996 identification of *NOTCH3* as the causative gene (Joutel et al., 1996). CADASIL is the most common Mendelian cerebral small-vessel disease, with a prevalence of approximately two to five per hundred thousand in European populations and with a phenotype that includes migraine with aura in the third and fourth decades, transient ischemic attacks and lacunar strokes in the fifth and sixth decades, subcortical vascular dementia in the sixth and seventh decades, and a confluent leukoencephalopathy on MRI that involves the anterior temporal lobes, the external capsule, and the periventricular white matter in a stereotyped distribution that is essentially pathognomonic in the right clinical context. The molecular biology of CADASIL has been characterized over three decades: the pathogenic *NOTCH3* mutations are missense substitutions that alter the number of cysteines in the EGF-like repeats of the extracellular domain, with the consequence that NOTCH3 is misfolded, accumulates in the vessel wall as granular osmiophilic material (GOM), and produces a degeneration of vascular smooth muscle cells and pericytes that progresses across the adult life course.

The 2009 identification of *HTRA1* as the causative gene for CARASIL (cerebral autosomal-recessive arteriopathy with subcortical infarcts and leukoencephalopathy; Hara et al., 2009) added a second small-vessel-disease gene to the catalog. CARASIL is rarer than CADASIL, with onset typically in the third and fourth decades, alopecia and spondylosis as extra-cerebral features, and a clinical course similar to CADASIL but more aggressive. HTRA1 is a serine protease that regulates TGF-β signaling; pathogenic loss-of-function variants increase TGF-β bioavailability in the vessel wall and produce a fibrotic vasculopathy. The 2015 identification of heterozygous *HTRA1* variants as risk factors for late-onset cerebral small-vessel disease at intermediate effect sizes (Verdura et al., 2015) extended the gene into the polygenic architecture and supplied a clean example of the allelic-series principle: rare homozygous LOF causes CARASIL; rare heterozygous LOF confers high-intermediate risk; common variation at the locus contributes to polygenic SVD risk.

The *COL4A1* and *COL4A2* genes, encoding the two alpha chains of type IV collagen that compose the principal collagen scaffold of the vascular basement

membrane, were identified as causative genes for a spectrum of cerebral vasculopathies including HANAC (hereditary angiopathy with nephropathy, aneurysms, and muscle cramps), porencephaly, and adult-onset cerebral small-vessel disease (Gould et al., 2005, 2006; Plaisier et al., 2007). The pathogenic variants are missense substitutions in the triple-helical Gly-X-Y repeat that disrupt collagen IV trimerization and produce a fragile basement membrane susceptible to mechanical and oxidative damage. The COL4A1/A2 spectrum supplies the third major Mendelian SVD axis after CADASIL and CARASIL and locates the load-bearing biology in the extracellular matrix of the vessel wall rather than in the cellular components of the wall itself.

The 2007 identification of *TREX1* as the causative gene for retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S; Richards et al., 2007) supplied an interferon-mediated mechanism of cerebrovascular damage. TREX1 is a 3′-to-5′ exonuclease that degrades cytoplasmic single-stranded DNA; loss of function produces cytoplasmic DNA accumulation, cGAS-STING activation, type-I interferon production, and an interferonopathy that targets retinal and cerebral microvasculature. The TREX1 mechanism is mechanistically distinct from the NOTCH3, HTRA1, and COL4A1/A2 mechanisms and supplies a fourth axis — the cytoplasmic-DNA-innate-immunity axis — through which the vascular genome can fail.

The 2018 identification of *CTSA* as the causative gene for CARASAL (cathepsin-A-related arteriopathy with strokes and leukoencephalopathy; Bugiani et al., 2016) and the well-established role of *GLA* (Fabry disease) and *MTHFR* (hyperhomocysteinemia) in cerebrovascular phenotypes complete the high-penetrance Mendelian core of the cerebrovascular genome.

2.3 The Cerebrovascular GWAS Era (2007–2026)

The genome-wide association study era for cerebrovascular phenotypes began with the 2007 deCODE study of intracranial aneurysm and the subsequent METASTROKE collaborations that identified common-variant loci for ischemic stroke, hemorrhagic stroke, and white-matter hyperintensities. The most recent ISGC meta-analysis (Mishra et al., 2022) reports more than 60 loci for ischemic stroke; the white-matter hyperintensity GWAS (Sargurupremraj et al., 2020) reports 27 loci, several of which overlap with the AD GWAS catalog including *EFEMP1* (a basement-membrane gene), *PITX2* (cardiac development and atrial fibrillation), *FOXF2* (forkhead transcription factor with cerebrovascular expression), and *COL4A2* (the collagen gene whose rare variants cause Mendelian SVD).

The cell-type expression of the cerebrovascular GWAS loci has been characterized in the human single-cell vasculature atlases (Yang et al., 2022; Garcia et al., 2022) and shows the expected enrichment in endothelial and pericyte populations rather than in microglia and astrocytes. The cell-type-resolved expression of the AD GWAS loci, by contrast, shows strong microglial enrichment (Novikova et al., 2021) but with a non-trivial vascular component that has often been overlooked: *PICALM* is expressed at substantial levels in brain endothelium and contributes to LRP1-mediated amyloid clearance at the vascular interface; *CLU* is expressed by endothelial cells and functions in amyloid clearance; *BIN1* is expressed in endothelium and pericytes in addition to microglia; *CR1* and *ABCA7* both have vascular expression components.

2.4 The Imaging-Genetics Era

The cohort-scale imaging-genetics studies of the past decade have produced the principal in vivo evidence for the genetic architecture of the cerebrovascular phenotype. The UK Biobank imaging cohort (Smith et al., 2021; Sargurupremraj et al., 2020) has supplied common-variant association data for white-matter hyperintensity volume, perivascular space burden, microbleed count, and arterial stiffness. The Framingham Heart Study Offspring Cohort has supplied longitudinal data on the same phenotypes with adult-life follow-up that extends to dementia conversion. The ENIGMA-CSVD consortium has integrated cerebrovascular imaging across multiple international cohorts. The Montagne–Zlokovic dynamic contrast-enhanced MRI studies have provided the in vivo characterization of regional BBB permeability across the adult lifespan and across APOE genotype, with the principal finding that APOE4 carriers exhibit hippocampal BBB permeability elevations that precede amyloid accumulation by a decade or more.

2.5 Methodology of the Present Dissertation

The dissertation employs an integrative methodology that combines five sources of evidence. First, the Mendelian-genetics catalog from OMIM and ClinVar is used to identify the high-penetrance core of the cerebrovascular genome. Second, the cerebrovascular and stroke GWAS meta-analyses are used to identify the common-variant architecture. Third, the human vasculature single-cell expression atlases (Yang et al., 2022; Garcia et al., 2022) are used to assign each gene to its principal vascular cell type of expression. Fourth, the imaging-genetics literature is used to validate the in vivo phenotypic relevance of each gene. Fifth, the Phase 0/I/II/III framework developed across the Collapse trilogy and its companion volumes is used to assign each gene to its phase of load-bearing. Genes are then organized into chapters by mechanism and by cellular substrate, with phase-spanning genes (principally APOE and the matrix-metalloproteinase family) treated as integrative nodes in Chapter IX.

The dissertation does not attempt a formal systematic review or meta-analysis. Its contribution is the construction of an integrative analytical schema centered on the vascular dimension as Phase 0 of late-onset neurodegeneration and the mapping of the existing cerebrovascular genome onto that schema.

3. Chapter I — Phase 0: The Vascular Architecture as a Temporal Stage Prior to Bioenergetic Ignition

3.1 The Temporal-Phase Framework and Its Gap

The temporal-phase framework articulated in the Collapse trilogy and elaborated in the Genetic Architecture dissertation organizes the natural history of late-onset Alzheimer's disease into three sequential cellular substrates. Phase I, beginning in the third and fourth decades, is anchored anatomically in the locus coeruleus and the noradrenergic projection system, biochemically in the NAD□ axis and the PARP-1/SARM1/NMNAT2 cassette, and pharmacologically in the mitochondrial quality-control machinery (PINK1/Parkin, OPA1, mitofusin biology). Phase II, beginning in the fifth and sixth decades, is anchored anatomically in the hippocampus and the medial temporal cortex, cellularly in the microglial transition from the Butovsky homeostatic signature to the disease-associated microglia phenotype, and biochemically in the TREM2-PI3K-AKT-mTOR substrate-bridge and the lipid-droplet-accumulating microglial phenotype. Phase III, beginning in the seventh decade and continuing through the ninth, is anchored anatomically in the cortex and the parvalbumin-positive interneuron populations, cellularly in the perineuronal-net substrate and the matrix-metalloproteinase-9-driven matrix collapse, and biochemically in the gamma-frequency-drive failure that follows PV+ interneuron disinhibition.

The framework is satisfying because it specifies, at each phase, the cellular substrate, the molecular effectors, and the anatomical location of the load-bearing biology, and because it supplies the natural-history scaffolding within which the genetic architecture can be read as a phase-resolved structure. The framework is incomplete, however, because it begins at the locus coeruleus and treats the cerebrovasculature as either absent or downstream. The Montagne–Zlokovic imaging data force the reading that hippocampal blood-brain-barrier permeability begins to rise in the fifth decade and is detectable by DCE-MRI in cognitively normal adults whose CSF amyloid and tau measurements are within normal range. The Wyss-Coray–Yousef plasma proteomics data force the reading that soluble VCAM-1 begins to rise in plasma in the fourth decade and reaches concentrations at the brain endothelium sufficient to induce endothelial activation by the fifth decade. The cerebrovascular MRI data force the reading that white-matter hyperintensities begin

to accumulate in the fifth decade and are detectable on imaging in the sixth decade across the adult population. These three pieces of evidence converge on the finding that a vascular event is detectable before the canonical Phase I event of locus-coeruleus bioenergetic ignition, and that the vascular event has a measurable trajectory across the adult life course.

3.2 The Definition of Phase 0

Phase 0 is defined, in the present framework, as the temporal stage that begins in the third decade and extends through the fifth, in which the cerebrovascular substrate accumulates the cellular and molecular changes that condition the subsequent entry into Phase I. The substrate of Phase 0 is the cerebrovascular cellular complex — brain endothelial cells, pericytes, vascular smooth muscle cells, perivascular macrophages, and the basement membrane that anchors the astrocytic endfoot. The proximate molecular events of Phase 0 are: (1) the age-dependent reduction in claudin-5 expression at the brain endothelium, with consequent paracellular permeability increase; (2) the age-dependent reduction in pericyte coverage of the brain capillary, with consequent loss of pericyte-derived PDGF-B / PDGFRβ support of endothelial integrity; (3) the age-dependent reduction in MFSD2A-mediated DHA-lysophosphatidylcholine import into the endothelial cell, with consequent permissive lipid composition for caveolar transcytosis; (4) the age-dependent rise in VCAM-1 expression at the brain endothelium under the systemic plasma-protein milieu, with consequent firm adhesion of α4β1-expressing aged monocytes; (5) the age-dependent reduction in LRP1 expression at the abluminal membrane and rise in RAGE expression at the luminal membrane, with consequent reversal of net amyloid-β transport from efflux to influx; and (6) the age-dependent reduction in AQP4 polarization at the astrocytic endfoot, with consequent compromise of the glymphatic clearance flow that maintains parenchymal solute homeostasis. Each of these six events has a genetic substrate, and each is heritable, modifiable, and pharmacologically tractable.

The natural-history sequence of Phase 0 begins at the tight junction in the third decade with the gradual decline of claudin-5 expression that the imaging-genetics data document, proceeds through the pericyte-coverage decline of the fourth decade that the Zlokovic-Montagne sPDGFRβ biomarker quantifies, accelerates in the fifth decade with the VCAM-1 induction that the Yousef plasma-proteomics data document, and consolidates in the sixth decade with the LRP1-to-RAGE transporter

shift that the Deane–Zlokovic mechanistic studies document. The locus-coeruleus bioenergetic ignition of Phase I is, on this account, partly an event of vascular substrate failure: the bioenergetic vulnerability of LC neurons is real, but the trigger for the ignition is the vascular substrate that fails to maintain the metabolic supply on which LC neurons depend.

3.3 The Cellular Geometry of Phase 0

The cerebrovascular substrate of Phase 0 differs from the substrates of Phases I–III in three respects that are decisive for the genetic architecture. First, the substrate is anatomically distributed: the cerebrovasculature is not a single nucleus or a single cortical region but a network that spans the entire brain, with the cumulative capillary length in a single human brain approaching four hundred miles and with no cortical neuron more than approximately twenty micrometers from the nearest capillary lumen. The genes that load-bear Phase 0 are therefore expressed in a cellular compartment whose anatomy is global rather than regional, and the phenotype of Phase 0 dysfunction is the integrated result of many small distributed failures rather than the punctate failure of a single nucleus.

Second, the substrate is mechanistically responsive to systemic risk factors in a manner that the parenchymal substrates of Phases I–III are not. The brain endothelium is the cellular interface at which midlife hypertension, dyslipidemia, hyperglycemia, hyperhomocysteinemia, chronic systemic inflammation, sleep apnea, and the aged plasma proteome impinge on the central nervous system. The genes that load-bear Phase 0 are therefore the genes whose effects compound with the lifetime cumulative vascular-risk-factor exposure, and the phenotype is the integrated genotype-by-environment interaction across forty years of adult life rather than the cell-autonomous failure of a vulnerable neuronal population.

Third, the substrate is bidirectionally permeable to the genetic architecture of cardiovascular disease, type 2 diabetes, and hypertension. The cerebrovascular GWAS catalog overlaps substantially with the coronary artery disease GWAS catalog, with the stroke GWAS catalog, and with the hypertension GWAS catalog, and the genes that load-bear Phase 0 are therefore not purely "AD genes" but are "vascular genes" whose AD phenotype is one expression among several. The implication is that the phase-zero substrate is the substrate at which the long-recognized shared genetic architecture of cardiometabolic and neurodegenerative disease is best read, and the

genes that emerge from the present analysis will be the same genes that emerge from a cardiovascular analysis read with a brain-endothelial lens.

3.4 The Question of Causal Order

The phase-zero framework does not assert that the vascular event is *the* causal initiator of late-onset neurodegeneration in every patient. The Mendelian forms of AD (APP and PSEN1/2 mutations), of FTD (MAPT, GRN, C9ORF72), of PD (SNCA, LRRK2), and of ALS (SOD1, TARDBP, C9ORF72) can initiate cellular trajectories that produce disease without requiring a Phase 0 substrate failure, and the parenchymal trajectory in these monogenic forms may outrun any vascular contribution. What the framework asserts is that in the typical sporadic late-onset patient — the 65-to-90-year-old with mixed pathology at autopsy, with midlife vascular risk factors, with cerebral microbleeds on MRI, and without a high-penetrance Mendelian variant — Phase 0 is load-bearing, the Phase 0 substrate has been compromised for two to four decades before clinical presentation, and the genes that load-bear Phase 0 have been silently selecting the trajectory for that period. The phase-zero framework supplies the genetic architecture of this period and the molecular targets that intervention during it would require.

4. Chapter II — The Tight Junction Genome and the Paracellular Partition

4.1 The Tight Junction Complex as a Genetic Unit

The tight junction complex of the brain endothelial cell is the molecular substrate of paracellular impermeability, and it is the substrate whose age-dependent failure constitutes the proximate molecular event of blood-brain-barrier compromise. The complex comprises three classes of transmembrane proteins — the claudin family (principally CLDN5 with smaller contributions from CLDN3, CLDN12, and CLDN25), the MARVEL-domain proteins (occludin / OCLN, MARVELD2 / tricellulin, MARVELD3), and the immunoglobulin-superfamily junctional adhesion molecules (JAM-A / F11R, JAM-B / JAM2, JAM-C / JAM3, CAR / CXADR, ESAM) — anchored to the actin cytoskeleton through the cytoplasmic zonula occludens scaffold proteins (TJP1 / ZO-1, TJP2 / ZO-2, TJP3 / ZO-3) and to intracellular signaling through cingulin (CGN), paracingulin (CGNL1), and the Crumbs polarity complex (CRB3, PALS1 / MPP5, PATJ / INADL).

Each component is genetically encoded, and each is a candidate for inherited variation that compromises the integrity of the complex. The dissertation treats the principal load-bearing genes in turn.

4.2 CLDN5 and the Paracellular Determinant of BBB Selectivity

CLDN5 (chromosome 22q11.21) encodes claudin-5, the principal molecular determinant of paracellular impermeability at the brain microvascular endothelium. Genetic deletion of *Cldn5* in mice produces a size-selective opening of the barrier to molecules below approximately 800 daltons within the first day of life and is incompatible with postnatal survival (Nitta et al., 2003). Conditional postnatal deletion of *Cldn5* in adult mice produces depressive and anxiety phenotypes and a leak of small molecules from the systemic compartment to the brain that has been mechanistically implicated in mood disorder (Menard et al., 2017). The age-dependent reduction in *CLDN5* expression at the human brain capillary, documented by Montagne, Zlokovic, and colleagues across the adult lifespan, is the proximate molecular event by which paracellular permeability increases with age.

The 22q11.21 chromosomal locus that contains *CLDN5* is the same locus deleted in DiGeorge syndrome (22q11.2 deletion syndrome), and the schizophrenia and cognitive-impairment phenotypes of DiGeorge syndrome have been mechanistically attributed in part to *CLDN5* haploinsufficiency (Greene et al., 2018). The common-variant architecture of *CLDN5* has not been extensively characterized in AD GWAS to date, but the gene's regulatory architecture is a candidate substrate for the inherited variation that the imaging-genetics literature has identified at the white-matter-hyperintensity and BBB-permeability phenotypes. The pharmacological reactivation of *CLDN5* expression — through inhibitors of the histone deacetylases that suppress the gene under inflammatory conditions — is in early preclinical development as a Phase-0-targeted intervention.

4.3 OCLN, the JAM Family, and the Tricellular Junction

OCLN (chromosome 5q13.2) encodes occludin, a MARVEL-domain transmembrane protein that contributes to tight-junction stability through phosphorylation-regulated interactions with the ZO scaffold. Occludin is not required for tight-junction formation — *Ocln*-null mice are viable and form intact tight junctions — but it is required for the regulated permeability of the junction under physiological stress, and its phosphorylation state regulates the leak of plasma constituents into the parenchymal compartment. Pathogenic variants in *OCLN* cause band-like calcification with simplified gyration and polymicrogyria (BLC-PMG, OMIM 251290; O'Driscoll et al., 2010), an autosomal-recessive cerebral developmental disorder with prominent vascular features that includes pseudo-TORCH-syndrome-like calcifications and that locates *OCLN* in the Mendelian core of the cerebrovascular genome.

The JAM family — *F11R* (JAM-A), *JAM2* (JAM-B), *JAM3* (JAM-C) — contributes to tight-junction stability and to leukocyte transmigration regulation. Pathogenic loss-of-function variants in *JAM3* cause hemorrhagic destruction of the brain with subependymal calcification and cataracts (Mochida et al., 2010), a Mendelian cerebrovascular phenotype that further locates the tight-junction-and-adhesion gene set in the high-penetrance core.

4.4 TJP1, TJP2, and the Cytoplasmic Scaffold

TJP1 (ZO-1, chromosome 15q13.1) and *TJP2* (ZO-2, chromosome 9q21.11) encode the cytoplasmic scaffold proteins that anchor the transmembrane tight-junction components to the actin cytoskeleton and to the intracellular signaling apparatus. The ZO proteins are essential for tight-junction integrity, and their reduced expression at the aged brain endothelium has been documented in multiple cohorts. Pathogenic variants in *TJP2* cause progressive familial intrahepatic cholestasis (PFIC4, OMIM 615878) and hypercholanemia, syndromes whose principal phenotype is hepatic but whose cerebrovascular sequelae have been documented in case reports. The common-variant architecture of *TJP1* and *TJP2* in the cerebrovascular context has not been extensively characterized, but the gene set is a candidate substrate for the inherited variation that the imaging-genetics literature implicates.

4.5 The Tight-Junction Gene Set as a Phase 0 Substrate

The tight-junction gene set is, in the framework of the present dissertation, the molecular substrate of the paracellular component of Phase 0. The genes load-bear the integrity of the paracellular partition between the systemic and the parenchymal compartments, and their failure under the cumulative oxidative and inflammatory stress of the adult life course is the proximate molecular event by which the BBB compromises with age. The genes are therefore Phase 0 genes in the strict sense: their effects are most consequential before the entry into Phase I, and their pharmacological protection during Phase 0 would be expected to delay the entry into the parenchymal cascade. The candidate intervention surface includes claudin-5-promoting agents (HDAC inhibitors, β-catenin pathway modulators), JAM-stabilizing agents (under preclinical development), and the broader class of endothelial-protective therapies that act through the upstream Wnt-β-catenin and Hedgehog signaling axes that maintain the tight-junction transcriptional program.

5. Chapter III — The Pericyte–Endothelial Axis: PDGFB, PDGFRB, NOTCH3, HTRA1, FOXF2

5.1 The Pericyte as a Vascular Cell Type

The pericyte is the contractile mural cell embedded in the basement membrane immediately adjacent to the brain endothelial cell and connected to it through peg-and-socket junctions and through soluble paracrine signaling. The pericyte-to-endothelial cell ratio in the brain capillary is approximately one-to-three, the highest of any capillary bed in the body, and the pericyte covers approximately thirty percent of the capillary surface area in cortical gray matter. The pericyte contributes to capillary blood flow regulation through its contractile state, to capillary stability through its paracrine support of endothelial tight junctions, to immune surveillance through its expression of pattern-recognition receptors, and to waste clearance through its participation in the perivascular drainage pathway. The pericyte is the cellular component of the neurovascular unit whose age-dependent loss has been most precisely quantified by the Zlokovic program and whose contribution to BBB integrity is most directly attested by the genetic models of pericyte ablation.

5.2 PDGFB and PDGFRB: The Cellular Recruitment Axis

PDGFB (chromosome 22q13.1) encodes the B chain of platelet-derived growth factor, secreted as a homodimeric PDGF-BB ligand by brain endothelial cells. *PDGFRB* (chromosome 5q32) encodes the β subunit of the cognate receptor, expressed on pericytes and on a subset of vascular smooth muscle cells. The PDGFB-PDGFRB signaling axis is required for the recruitment, survival, and maintenance of pericytes at the brain capillary; genetic ablation of either ligand or receptor produces a phenotype of pericyte loss, BBB breakdown, microvascular degeneration, and cognitive impairment in mice (Lindahl et al., 1997; Armulik et al., 2010; Bell et al., 2010). Pericyte-deficient mice exhibit accelerated amyloid-β accumulation in the parenchyma, secondary to the LRP1 reduction at the brain endothelium that follows the loss of pericyte support (Sagare et al., 2013). The axis is, in the framework of the present chapter, the principal Phase 0 substrate for the maintenance of pericyte coverage across the adult life course.

Pathogenic heterozygous variants in *PDGFRB* cause infantile myofibromatosis (OMIM 228550), Kosaki overgrowth syndrome (OMIM 616592), and the recently characterized primary familial brain calcification syndromes (OMIM 615007; Nicolas et al., 2013), with the latter producing basal ganglia, thalamic, and cerebellar calcifications and progressive neurological decline. The *PDGFRB*-driven calcification syndromes locate the gene in the Mendelian cerebrovascular core and supply a high-penetrance allelic series whose effector pathway is precisely the pericyte axis that the Zlokovic two-hit framework develops at lower penetrance.

The biomarker correlate of pericyte injury — soluble PDGFRβ (sPDGFRβ), the shed extracellular domain released into CSF as pericytes are damaged — was established by the Montagne group as a quantitative biomarker of pericyte injury that correlates with hippocampal BBB permeability on DCE-MRI and with subsequent cognitive decline. CSF sPDGFRβ elevation is detectable in cognitively normal APOE4 carriers in the fifth and sixth decades, in advance of amyloid PET positivity, and is the principal candidate Phase 0 biomarker for the pericyte-substrate component of the framework.

5.3 NOTCH3 and CADASIL

NOTCH3 (chromosome 19p13.12) encodes the third Notch receptor, expressed principally in vascular smooth muscle cells and pericytes. NOTCH3 is a single-pass transmembrane receptor with an extracellular domain composed of thirty-four EGF-like repeats and three Notch/Lin-12 repeats, a transmembrane segment, and an intracellular Notch-domain that translocates to the nucleus on receptor activation to regulate target-gene transcription. Pathogenic variants in *NOTCH3* cause CADASIL, the most common Mendelian cerebral small-vessel disease, with prevalence in European populations of approximately two to five per hundred thousand.

The pathogenic variants are missense substitutions that alter the number of cysteines in the EGF-like repeats — the canonical CADASIL mutation introduces or removes a single cysteine, with the consequence that the three pairs of cysteines that ordinarily form intra-repeat disulfide bonds are disrupted and an unpaired cysteine is left at the protein surface (Joutel et al., 1996, 1997). The unpaired cysteine produces intermolecular disulfide bonds that misfold the protein, drive its accumulation in the vessel wall as granular osmiophilic material (GOM), and produce the progressive degeneration of vascular smooth muscle cells and pericytes

that defines the disease.

The CADASIL phenotype begins in the third and fourth decades with migraine with aura, progresses through transient ischemic attacks and lacunar strokes in the fifth and sixth decades, and terminates in subcortical vascular dementia in the sixth and seventh decades. The imaging signature is a confluent leukoencephalopathy that involves the anterior temporal lobes, the external capsule, and the periventricular white matter in a stereotyped distribution. The CADASIL natural history is therefore a *compressed* and *accelerated* version of the sporadic small-vessel-disease trajectory that the typical 80-year-old AD patient exhibits, and the gene-effector axis is the same.

The common-variant architecture of *NOTCH3* has been examined in the cerebrovascular GWAS catalog (Sargurupremraj et al., 2020), and modifier variants at the locus contribute to the burden of white-matter hyperintensities in adult populations. Genome-wide sequencing studies have identified *NOTCH3* cysteine-altering variants at population frequencies up to 1 in 300 in the general adult population (Rutten et al., 2016), with substantial variability in penetrance and age of onset. The expanded frequency reading converts NOTCH3 from a rare Mendelian gene into an intermediate-frequency contributor to adult cerebral small-vessel disease, and the implications for population-scale screening and intervention are non-trivial.

5.4 HTRA1 and CARASIL

HTRA1 (chromosome 10q26.13) encodes high-temperature requirement A1, a secreted serine protease that regulates TGF-β bioavailability in the extracellular space through cleavage of TGF-β-binding proteins and through direct cleavage of TGF-β family ligands. The HTRA1-TGF-β axis is the principal load-bearing axis for the maintenance of vessel-wall homeostasis in the cerebral small-vessel circulation, and its dysregulation produces a fibrotic vasculopathy with a phenotype similar to but more aggressive than CADASIL.

Pathogenic homozygous loss-of-function variants in *HTRA1* cause CARASIL (Hara et al., 2009), an autosomal-recessive small-vessel disease with onset in the third and fourth decades that includes alopecia and spondylosis as extra-cerebral features alongside the central cerebrovascular phenotype. Pathogenic heterozygous loss-of-function variants in *HTRA1* — initially thought to be benign carrier states —

were subsequently identified as risk factors for late-onset cerebral small-vessel disease at intermediate effect sizes (Verdura et al., 2015), with carrier frequencies in the range of 1 in 1,000 to 1 in 300 in European populations. The heterozygous *HTRA1* finding is one of the most important recent results in the cerebrovascular genetics literature because it establishes a clean allelic-series principle: homozygous LOF causes Mendelian CARASIL; heterozygous LOF confers intermediate risk for late-onset cerebral SVD; and common variation at the locus contributes to polygenic SVD risk at small effect sizes.

The *HTRA1* gene is, additionally, the strongest common-variant association in the age-related macular degeneration (AMD) GWAS catalog (Dewan et al., 2006; Yang et al., 2006), with the principal AMD risk variant in linkage disequilibrium with variants in the adjacent *ARMS2* gene. The AMD-CARASIL-cerebral-SVD connection establishes *HTRA1* as a master vascular-protease gene whose dysregulation produces age-related microvascular disease across multiple tissues, and the locus is a candidate Phase-0-targeted intervention surface.

5.5 FOXF2, FOXC1, and the Forkhead Transcription Factor Axis

FOXF2 (chromosome 6p25.3) and *FOXC1* (chromosome 6p25.3) encode forkhead transcription factors expressed in pericytes and in vascular smooth muscle cells. Both genes are within the 6p25.3 chromosomal region whose hemizygous loss is associated with anterior-segment dysgenesis, cerebrovascular abnormalities, and cognitive impairment. The white-matter-hyperintensity GWAS (Sargurupremraj et al., 2020) identified a strong common-variant signal at 6p25.3 that has been attributed to *FOXF2* on the basis of its pericyte-expressed transcription-factor function, and the same locus has been associated with stroke risk in the ISGC meta-analyses. *FOXF2* and *FOXC1* are therefore the canonical forkhead transcription-factor anchors of the polygenic cerebrovascular architecture, and they connect the pericyte-substrate biology of Phase 0 to the broader forkhead-regulated transcriptional program that maintains the cerebrovascular cellular identity.

5.6 The Pericyte–Endothelial Axis as a Phase 0 Substrate

The pericyte–endothelial axis is, in the framework of the present dissertation, the principal cellular substrate of Phase 0. The genes that load-bear the axis — *PDGFB*, *PDGFRB*, *NOTCH3*, *HTRA1*, *FOXF2*, *FOXC1* — together specify the inherited variation that determines pericyte coverage of the brain capillary at the beginning of Phase 0, the rate of pericyte loss across the adult life course, the severity of consequent endothelial-tight-junction failure, and the timing of entry into Phase I. The biomarker correlate (CSF sPDGFRβ) and the imaging correlate (DCE-MRI BBB permeability) supply the in vivo readouts of the substrate, and the Mendelian small-vessel-disease genes supply the high-penetrance allelic anchors of the architecture. The pericyte axis is the substrate at which Phase 0 intervention is most cleanly conceptualized, and the candidate intervention surface includes pericyte-targeted PDGFRβ agonists, NOTCH3-targeting antisense oligonucleotides (in development for CADASIL), and the broader class of TGF-β-axis modulators that the HTRA1 mechanism implicates.

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6. Chapter IV — Transcytosis, Transporter Geometry, and the Curated Interface

6.1 The Transcytotic Suppression Machinery

The brain endothelial cell suppresses vesicular transcytosis at baseline through the action of *MFSD2A* (chromosome 1p34.2), a membrane transporter that imports docosahexaenoic acid lysophosphatidylcholine (DHA-LPC) into the endothelial cell and maintains a lipid composition incompatible with caveolar formation (Nguyen et al., 2014; Andreone et al., 2017). The MFSD2A-mediated DHA-LPC import is the principal route by which DHA, an omega-3 polyunsaturated fatty acid required for neuronal membrane fluidity and synaptic function, crosses the BBB into the central nervous system. The transporter is selectively expressed on brain endothelial cells and is required for both the lipid composition that suppresses caveolar transcytosis and the DHA supply that the parenchyma requires.

Pathogenic loss-of-function variants in *MFSD2A* cause a severe autosomal-recessive microcephaly syndrome (Guemez-Gamboa et al., 2015), with progressive neurodegeneration that establishes the gene as a Mendelian

developmental neurological gene. The age-dependent reduction in *MFSD2A* expression at the human brain endothelium has been documented and is the molecular substrate of the increased transcytotic activity observed in the aged brain, on which the VCAM-1 mechanism developed in Chapter V operates. The gene's common-variant architecture is a candidate Phase 0 contributor that has not been extensively characterized in the AD GWAS literature to date.

6.2 The Glucose Transporter Axis: SLC2A1 (GLUT1)

SLC2A1 (chromosome 1p34.2) encodes glucose transporter 1 (GLUT1), the principal glucose transporter at the brain capillary endothelium and at the erythrocyte. GLUT1 executes the bidirectional facilitated diffusion of glucose between blood and brain across the concentration gradient established by parenchymal glucose consumption. Pathogenic heterozygous loss-of-function variants in *SLC2A1* cause GLUT1 deficiency syndrome (De Vivo et al., 1991), with the principal phenotype an epileptic encephalopathy of infancy that responds to ketogenic diet and that supplies the cleanest human evidence that brain glucose transport is a metabolically load-bearing function.

The age-dependent reduction in *SLC2A1* expression at the brain endothelium in human autopsy studies, with acceleration in Alzheimer's disease (Winkler et al., 2015), is the molecular substrate of the temporoparietal hypometabolism that is one of the most reliable FDG-PET biomarkers of early AD. The GLUT1-reduction mechanism reframes FDG-PET hypometabolism as a vascular-transporter event rather than a parenchymal-consumption event in significant measure, and it locates *SLC2A1* in the Phase 0 substrate as the molecular determinant of brain glucose supply across the adult life course. The conditional postnatal deletion of *Slc2a1* in mouse brain endothelium produces Alzheimer-like cognitive deficits in the absence of parenchymal amyloid or tau pathology (Winkler et al., 2015), supplying the clean experimental demonstration that endothelial glucose transport is a load-bearing function for cognition.

6.3 The LRP1-RAGE-P-Glycoprotein Transporter Triangle

LRP1 (chromosome 12q13.3) encodes low-density-lipoprotein-receptor-related protein 1, a multi-ligand endocytic receptor expressed at the abluminal membrane of the brain endothelial cell. LRP1 binds free amyloid-β with submicromolar affinity, transcytoses the peptide across the endothelial cell to the luminal membrane, and releases it into the systemic circulation for hepatic clearance (Deane et al., 2004). The age-dependent reduction in *LRP1* expression at the abluminal brain endothelium is the molecular substrate of the reduced amyloid-β efflux that contributes to parenchymal amyloid accumulation in aging and in AD.

AGER (chromosome 6p21.32) encodes the receptor for advanced glycation end products (RAGE), expressed at the luminal membrane of the brain endothelial cell. RAGE binds circulating amyloid-β and transcytoses it into the parenchymal compartment, executing the reverse of the LRP1 pathway (Deane et al., 2003). The age-dependent rise in *AGER* expression at the luminal brain endothelium is the molecular substrate of the increased amyloid-β influx that contributes to parenchymal accumulation. The LRP1-to-RAGE shift — efflux down, influx up — is the proximate molecular event by which the brain endothelium's net amyloid handling reverses with age.

ABCB1 (chromosome 7q21.12) encodes P-glycoprotein (P-gp), an ATP-dependent efflux transporter at the luminal membrane of the brain endothelial cell. P-gp executes the active efflux of xenobiotics and of a subset of endogenous metabolites against their concentration gradient and contributes as a secondary substrate to amyloid-β efflux. The age-dependent reduction in *ABCB1* expression has been documented at the human brain endothelium (van Assema et al., 2012) and contributes to parallel mechanisms of vascular amyloid accumulation. *ABCG2* (chromosome 4q22.1) encodes breast cancer resistance protein (BCRP), an additional efflux transporter at the luminal membrane with overlapping substrate specificity and a similar age-dependent reduction.

The four-gene transporter triangle — *LRP1*, *AGER*, *ABCB1*, *ABCG2* — together specifies the vascular geometry of amyloid handling at the BBB, and the inherited variation at these loci is a candidate Phase 0 contributor to the rate of parenchymal amyloid accumulation across the adult life course.

6.4 The Curated Interface as a Phase 0 Substrate

The transcytosis and transporter gene set is, in the framework of the present dissertation, the molecular substrate of the curated interface across which the brain reads its systemic environment. The genes load-bear the active selectivity of the BBB — the suppression of nonselective transcytosis (*MFSD2A*), the maintenance of glucose supply (*SLC2A1*), the directional handling of amyloid-β (*LRP1*, *AGER*, *ABCB1*, *ABCG2*) — and their age-dependent dysfunction is a load-bearing component of Phase 0. The candidate intervention surface includes MFSD2A-directed DHA-LPC supplementation (now in early clinical trial), RAGE antagonists (azeliragon and analogues, with mixed clinical results), and the broader class of endothelial-protective therapies that act through PPARγ and Nrf2 transcriptional programs that maintain the transporter expression patterns.

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7. Chapter V — The VCAM-1 / BACE2 Axis as the Integrative Vascular Gateway

7.1 VCAM-1 as the Aged-Plasma Gateway

VCAM1 (chromosome 1p21.2) encodes vascular cell adhesion molecule-1, an immunoglobulin-superfamily transmembrane glycoprotein expressed on activated endothelial cells. VCAM-1 is not expressed on resting vascular endothelium; its expression is induced by TNF-α, IL-1β, and other inflammatory cytokines through an NF-κB-dependent transcriptional program. The cognate ligand is the α4β1 integrin (very late antigen-4, VLA-4) expressed on lymphocytes, monocytes, eosinophils, and basophils, encoded by *ITGA4* (chromosome 2q31.3) and *ITGB1* (chromosome 10p11.22). VCAM-1 binding to α4β1 mediates the firm adhesion of leukocytes to activated endothelium that precedes their transendothelial migration into the underlying tissue.

The Wyss-Coray–Yousef program at Stanford established that soluble VCAM-1 (sVCAM-1) — the shed extracellular domain released from the surface of activated endothelium by metalloproteinase cleavage — is the single most age-upregulated soluble protein in mammalian plasma, with plasma sVCAM-1 concentrations rising by approximately tenfold across the adult mouse lifespan and by approximately fivefold across the adult human lifespan (Yousef et al., 2019). The Yousef paper went

further and established that aged plasma induces VCAM-1 expression on brain endothelial cells in young recipient mice, that brain endothelial VCAM-1 is necessary for the downstream effects of aged plasma on hippocampal microglia, on hippocampal neurogenesis, and on hippocampal-dependent cognition, and that pharmacological antibody blockade of VCAM-1 or tissue-specific conditional deletion of *Vcam1* in brain endothelial cells abolishes these effects. The brain endothelial VCAM-1 is thus the necessary mediator through which the aged systemic milieu transduces a parenchymal signal, and the molecule is a candidate therapeutic target for the prevention of brain aging.

The biomarker correlates of *VCAM1* in human AD are substantial and convergent. CSF sVCAM-1 is elevated in the preclinical, prodromal, and dementia stages of AD relative to age-matched cognitively normal controls. The CSF VCAM-1 elevation correlates with CSF total tau and phosphorylated tau, with cortical thinning on structural MRI, and with subsequent cognitive deterioration in longitudinal follow-up. The biomarker behavior of *VCAM1* is therefore not the behavior of a downstream marker of advanced disease but the behavior of an early-stage marker whose elevation precedes the parenchymal events of amyloid and tau accumulation in the natural history of the disease.

7.2 BACE2 as the Principal Sheddase of VCAM-1

BACE2 (chromosome 21q22.3) encodes β-site amyloid-precursor-protein-cleaving enzyme 2, a transmembrane aspartyl protease homologous to *BACE1* whose principal physiological function had remained partially obscure until the recent proteomic identification of VCAM-1 as its dominant substrate. BACE2 cleaves VCAM-1 in a manner analogous to BACE1's cleavage of amyloid precursor protein, releasing soluble VCAM-1 ectodomain into the extracellular space and leaving a membrane-bound C-terminal fragment.

The BACE2-VCAM-1 connection is, on its own, a remarkable convergence: the principal molecular gateway of aged-plasma toxicity to the brain is regulated by a member of the same enzyme family that produces amyloid-β, and the cleavage of VCAM-1 by BACE2 is the principal known physiological function of an enzyme whose pharmacological inhibition has been considered an adverse-effect liability for early BACE-inhibitor programs that lacked BACE1 selectivity. The pharmacological implication is that BACE2 inhibition would be expected to reduce VCAM-1 shedding,

with consequent membrane retention of full-length VCAM-1 and potentially enhanced firm-adhesion-mediated leukocyte recruitment at the brain endothelium. The BACE2-status of any candidate BACE inhibitor is therefore a Phase 0 question, and the selectivity profile of BACE inhibitors against BACE1 versus BACE2 is a pharmacological substrate of their cerebrovascular safety.

ADAM17 (chromosome 2p25.1) encodes a disintegrin and metalloproteinase 17, the secondary sheddase of VCAM-1 and of multiple other adhesion molecules and cytokine precursors. The age-dependent regulation of *ADAM17* expression is a load-bearing component of the inflammatory milieu, and the gene's pharmacological inhibition has been examined in inflammatory disease but not in AD specifically.

7.3 The VCAM-1 / BACE2 / α4β1 Axis as a Phase 0 Substrate

The *VCAM1* / *BACE2* / *ITGA4* / *ITGB1* / *ICAM1* / *ADAM17* gene complex is, in the framework of the present dissertation, the integrative gateway through which the aged systemic milieu is transduced into a parenchymal signal at the brain endothelium. The genes load-bear the cellular event by which the inflammatory plasma proteome is converted into the brain-endothelial-activation phenotype that produces the IL-6 and CCL2 secretion into the abluminal compartment, the firm adhesion of aged monocytes, the activation of perivascular macrophages, and the loss of the homeostatic microglial signature that the Homeostatic Microglial Collapse thesis identifies as the central event of Phase II. The VCAM-1 gateway is therefore both the *exit* of Phase 0 and the *entry* of Phase II — it is the Phase-0-to-Phase-II bridge in the present framework, and its genetic architecture supplies the molecular determinants of the rate of bridge transit.

The candidate intervention surface includes natalizumab (an anti-α4β1 antibody approved for multiple sclerosis whose repurposing for AD is under preclinical evaluation), anti-VCAM-1 antibodies (under development), BACE2-selective inhibitors (under exploration in the diabetes context, where BACE2 regulates β-cell function), and ADAM17 inhibitors (in oncology development with possible cerebrovascular indications).

8. Chapter VI — APOE Re-Read as a Vascular Master Variable

8.1 APOE as a Vascular Gene

APOE has the largest per-allele effect of any gene in the AD common-variant architecture, with the APOE4 allele conferring approximately 3-fold heterozygous and 12-to-14-fold homozygous odds ratios for late-onset AD. The conventional reading of the APOE effect locates its load-bearing biology in the astrocyte-and-microglial compartment of the parenchyma, with cholesterol distribution as the principal cellular function. The phase-zero reading developed in the present dissertation re-locates a substantial fraction of the APOE effect to the vascular substrate and identifies the cyclophilin-A / MMP-9 / claudin-5 pathway in pericytes as the proximate molecular substrate of APOE4-driven BBB breakdown.

The mechanism, characterized by the Bell–Sagare–Zlokovic program (Bell et al., 2012), proceeds as follows. APOE3 and APOE2 isoforms bind the LRP1 receptor on pericytes with high affinity, with the binding suppressing the *PPIA* (cyclophilin A) / NF-κB / *MMP9* signaling axis and maintaining the pericyte's homeostatic phenotype. APOE4 binds LRP1 with reduced affinity, with the consequence that the *PPIA-NF-κB-MMP9* axis is constitutively activated in APOE4-carrier pericytes, cyclophilin A is elevated, NF-κB is activated, and MMP-9 is secreted into the extracellular space. The secreted MMP-9 then degrades the tight-junction proteins of the adjacent endothelial cell — claudin-5, occludin, and ZO-1 — with the consequence that paracellular permeability rises and the BBB compromises. The APOE4 vascular phenotype is therefore a pericyte-mediated event whose proximate effector is MMP-9 and whose proximate target is the endothelial tight junction.

The Montagne–Zlokovic group has extended this molecular framework to a clinical population (Montagne et al., 2020) and demonstrated that APOE4 carriers exhibit hippocampal BBB breakdown years before they exhibit amyloid accumulation or cognitive symptoms, and that the BBB breakdown is the earliest detectable biomarker change in the APOE4 trajectory. The vascular phenotype of APOE4 is the *first* phenotype in the natural history of the carrier, not the last — and the conventional ordering, in which APOE4 is read as primarily a parenchymal gene with secondary vascular consequences, has had the temporal sequence backwards.

8.2 The PPIA-NF-κB-MMP9-CLDN5 Pathway as a Gene Module

The *PPIA* (chromosome 7p13), *MMP9* (chromosome 20q13.12), *TIMP3* (chromosome 22q12.3), *LRP1*, *CLDN5*, and *NFKB1* genes together constitute a coordinated functional module whose pharmacological state determines the rate of APOE4-driven BBB breakdown. *PPIA* encodes cyclophilin A, the principal effector of the APOE4-driven NF-κB activation in pericytes. *MMP9* encodes matrix metalloproteinase-9, the proximate protease that degrades the endothelial tight-junction proteins. *TIMP3* encodes tissue inhibitor of metalloproteinase 3, the principal physiological inhibitor of MMP-9; pathogenic variants in *TIMP3* cause Sorsby fundus dystrophy (OMIM 136900), a Mendelian retinal vascular disease that supplies a clean analogy to the cerebrovascular phenotype of MMP-9 hyperactivity. *LRP1* is the upstream receptor whose APOE-binding affinity determines the input to the pathway. *CLDN5* is the downstream target whose proteolytic degradation is the proximate molecular event of barrier compromise.

The pharmacological intervention surface at the module is substantial. Cyclosporine A is a cyclophilin A inhibitor that has been examined in murine models of APOE4-driven BBB breakdown with mechanistic confirmation of the pathway (Bell et al., 2012). Selective MMP-9 inhibitors are in clinical development for multiple indications. The TIMP-3-mimetic approach is in early preclinical exploration. The candidate Phase-0-targeted intervention in APOE4 carriers is the pharmacological suppression of the PPIA-MMP9 axis with cyclosporine analogues or selective MMP-9 inhibitors, with the candidate biomarker readout the normalization of CSF claudin-5 fragment levels and the in vivo readout the reduction of hippocampal BBB permeability on DCE-MRI.

8.3 The Three-Phase Mechanism Re-Read with a Vascular Prior

The companion volume on the Genetic Architecture of Neurodegeneration developed a three-phase mechanism of APOE in which APOE4 contributes to disease through three distinct phase-specific effects: lipid mishandling in Phase I, microglial-state collapse and the lipid-droplet-accumulating-microglia phenotype in Phase II, and complement-mediated PNN attack in Phase III. The phase-zero reading adds a fourth phase-specific effect — APOE4-driven pericyte and BBB collapse — and re-orders the temporal sequence such that the vascular effect is the *first* effect, beginning in the fourth decade and consolidating across the fifth, with the lipid-mishandling, microglial-state, and PNN effects accumulating downstream. APOE is, on this revised account, a *Phase-0-through-Phase-III* master variable whose pleiotropic mechanism touches every stage of the disease through a different effector at each stage, and whose dominant effect at any given moment in the patient's life course depends on which phase is most active.

The reading clarifies why no single mechanism of APOE4 has been resolved despite three decades of work: all of the mechanisms are real, and the gene's effect is the sum of contributions across phases, with the vascular contribution dominant in the third through fifth decades, the lipid and microglial contributions dominant in the fifth through seventh, and the PNN contribution dominant in the seventh and beyond. The reading also clarifies why ARIA risk is so disproportionate in APOE4/4 homozygotes: their Phase 0 vascular substrate is the most compromised, their CAA burden is the highest, and the amyloid-clearance event imposed by monoclonal antibody therapy destabilizes a vessel wall whose structural integrity has already been depleted by the lifetime PPIA-MMP9-CLDN5 attack.

9. Chapter VII — The Mendelian Cerebral Small-Vessel-Disease Genes

9.1 The Allelic-Series Architecture

The Mendelian cerebral small-vessel-disease genes — *NOTCH3* (CADASIL), *HTRA1* (CARASIL), *COL4A1* and *COL4A2* (HANAC and porencephaly), *TREX1* (RVCL-S), *CTSA* (CARASAL), *GLA* (Fabry), and the rarer *FOXC1*, *ABCC6*, and *MTHFR* axes — together constitute the high-penetrance anchor of the cerebrovascular genome and supply the cleanest evidence that the cerebrovasculature has a genome whose inherited variation produces late-onset cognitive impairment. Each gene exhibits an allelic-series architecture in which homozygous or compound-heterozygous loss-of-function produces a severe Mendelian phenotype, heterozygous loss-of-function produces an intermediate phenotype at intermediate population frequency, and common-variant noncoding variation contributes to polygenic risk at small effect sizes. The architecture is not unique to the SVD genes — it is the canonical architecture of complex-trait genetics across human biology — but it is unusually well-characterized in this gene set because the phenotype is imageable in vivo, biomarker-trackable in CSF and plasma, and clinically distinct enough to support strong pedigree studies.

9.2 NOTCH3 and the Cysteine-Series Mechanism

The CADASIL allelic series has been characterized in detail in §5.3. The expanded reading is that the *NOTCH3* cysteine-altering variants — which were once thought to be rare — are present at population frequencies up to 1 in 300 in the general adult population (Rutten et al., 2016), with substantial variability in penetrance and age of onset. The expanded frequency reading converts CADASIL from a rare-disease curiosity into an intermediate-frequency contributor to adult cerebral small-vessel disease, and the screening implications are non-trivial. A 1-in-300 carrier frequency for a fully penetrant SVD allele in the adult population is a substantial public-health load, and the disease-modification surface that exists — antisense oligonucleotide therapy targeting *NOTCH3* mRNA, in preclinical development by multiple groups — supplies a candidate intervention whose deployment will require population-scale screening.

9.3 HTRA1 and the Heterozygote-Risk Mechanism

The CARASIL allelic series has been characterized in §5.4. The heterozygous-LOF reading by Verdura and colleagues (2015) is one of the most important recent findings in the literature because it established that carrier status for a Mendelian-disease allele at *HTRA1* confers a substantial increment in late-onset cerebral SVD risk, with the carrier frequencies in the 1-in-1,000-to-1-in-300 range producing a non-trivial population-attributable risk. The same allelic-series principle has been extended to a small number of additional cerebrovascular Mendelian genes whose heterozygous carrier status confers intermediate risk for late-onset SVD; the candidate list includes *COL4A1*, *COL4A2*, *FOXC1*, and *TREX1*.

9.4 The COL4A1/A2 Basement-Membrane Axis

COL4A1 (chromosome 13q34) and *COL4A2* (chromosome 13q34) encode the two principal alpha chains of type IV collagen, the major collagen of the vascular basement membrane. The collagen IV trimer (typically α1α1α2) is the structural scaffold of the basement membrane, and its integrity is required for the mechanical stability of the vessel wall and for the maintenance of the abluminal compartment through which the perivascular drainage pathway operates. Pathogenic missense variants in the triple-helical Gly-X-Y repeat disrupt collagen trimerization and produce a fragile basement membrane susceptible to mechanical and oxidative damage (Gould et al., 2005, 2006; Plaisier et al., 2007).

The phenotypic spectrum of *COL4A1/A2* mutations is broad and includes prenatal porencephaly, perinatal intracerebral hemorrhage, childhood-onset hereditary angiopathy with nephropathy and muscle cramps (HANAC), adult-onset small-vessel disease, and an increased risk of spontaneous intracerebral hemorrhage at any age. The locus is also a strong common-variant association in the WMH GWAS catalog, and the basement-membrane substrate it specifies is the structural anchor of the vascular dimension that the present dissertation develops.

9.5 TREX1 and the Cytoplasmic-DNA-Innate-Immunity Axis

TREX1 (chromosome 3p21.31) encodes three-prime repair exonuclease 1, a 3′-to-5′ exonuclease that degrades cytoplasmic single-stranded DNA. Loss of function produces cytoplasmic DNA accumulation, cGAS-STING activation, type-I interferon production, and the interferonopathy phenotype of retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S; Richards et al., 2007). The TREX1 mechanism supplies the cytoplasmic-DNA-innate-immunity axis through which the vascular genome can fail and connects the cerebrovascular SVD phenotype to the broader type-I-interferonopathy spectrum (Aicardi-Goutières syndrome, systemic lupus erythematosus, chilblain lupus). The cGAS-STING axis has been independently implicated in the aging-microglial transition (Gulen et al., 2023), and the *TREX1* locus is therefore a candidate Phase-0-to-Phase-II bridge through the shared cGAS-STING signaling.

9.6 The Broader Mendelian SVD Catalog

The Mendelian SVD catalog includes additional genes that complete the high-penetrance core: *CTSA* (CARASAL, with the late-onset arteriopathy-and-leukoencephalopathy phenotype; Bugiani et al., 2016), *GLA* (Fabry disease, with the X-linked lysosomal storage disorder that produces a small-vessel arteriopathy; Brady et al., 1967), *MTHFR* (hyperhomocysteinemia, with intermediate-penetrance contribution to thrombotic and arteriopathic disease through homocysteine accumulation), *ABCC6* (pseudoxanthoma elasticum, with mineralization of vascular elastic fibers), and the rare *ITM2B* / *BRI2* variants that cause familial British and Danish dementias with cerebral amyloid angiopathy. The catalog spans multiple mechanistic axes — Notch signaling, TGF-β protease regulation, collagen-IV basement-membrane scaffolding, cytoplasmic-DNA innate immunity, lysosomal lipid storage, elastic-fiber mineralization, and homocysteine metabolism — and supplies the high-penetrance evidence that the vascular genome is structured, load-bearing, and pharmacologically tractable.

10. Chapter VIII — The Glymphatic Genome: AQP4, GFAP, and the Meningeal-Lymphatic Axis

10.1 The Glymphatic System as a Genetically Encoded Architecture

The glymphatic clearance pathway, characterized by the Nedergaard–Iliff program (Iliff et al., 2012; Xie et al., 2013), is the cerebrospinal-fluid-to-interstitial-fluid exchange system through which solute waste — including amyloid-β, tau, and metabolic byproducts — is cleared from the parenchymal compartment of the brain. The pathway depends on three genetically encoded components: the polarized expression of aquaporin-4 at the astrocytic endfoot membrane that contacts the basement membrane, the architectural integrity of the perivascular Virchow-Robin space along cortical and leptomeningeal arteries, and the meningeal lymphatic vessels that drain the perivenous outflow into the deep cervical lymph nodes. Each component has a genetic substrate, and each is a candidate Phase 0 contributor to the parenchymal trajectory.

10.2 AQP4 and the Polarization of the Astrocytic Endfoot

AQP4 (chromosome 18q11.2) encodes aquaporin-4, the principal water channel of the central nervous system, expressed selectively on astrocytic membranes with a strict polarization to the endfoot that contacts the basement membrane. The polarization is required for the directionality of the glymphatic flow — water and solutes enter the parenchyma along the periarterial space through AQP4 channels at the endfoot facing the artery, and water exits the parenchyma along the perivenous space through AQP4 channels at the endfoot facing the vein. The loss of AQP4 polarization, observed in aged human brain and accelerated in AD (Zeppenfeld et al., 2017), abolishes the directionality of the flow and reduces the clearance efficiency by an order of magnitude.

The *AQP4* gene has not been a strong AD GWAS hit at population frequencies, but the gene's regulatory architecture is a candidate substrate for the inherited variation that determines the rate of AQP4-polarization loss with age. The dystroglycan-dystrobrevin complex that anchors AQP4 at the endfoot membrane — encoded by *DTNA* (dystrobrevin α), *DTNB* (dystrobrevin β), *DAG1* (dystroglycan), and *SNTA1* / *SNTB1* / *SNTB2* (syntrophins) — is the structural substrate of AQP4 polarization, and pathogenic variants in these genes produce muscular dystrophies

with central nervous system involvement that includes glymphatic-relevant phenotypes.

10.3 The Astrocyte-Endfoot Axis

GFAP (chromosome 17q21.31) encodes glial fibrillary acidic protein, the principal intermediate filament protein of mature astrocytes and a load-bearing component of the astrocyte-endfoot cytoskeleton. Pathogenic variants in *GFAP* cause Alexander disease, an autosomal-dominant leukodystrophy with the principal phenotype the accumulation of Rosenthal fibers in astrocytes and the secondary phenotype white-matter degeneration with prominent vascular and clinical-neurological involvement. *MLC1* (chromosome 22q13.33) encodes a transmembrane protein selectively expressed at the astrocyte-endfoot membrane; pathogenic variants cause megalencephalic leukoencephalopathy with subcortical cysts (MLC), with the principal phenotype the accumulation of vacuolated myelin and the secondary phenotype a vascular-glial cytoarchitectural disturbance.

10.4 The Meningeal-Lymphatic Drainage Architecture

The 2015 identification of meningeal lymphatic vessels in the dural sinuses (Louveau et al., 2015; Aspelund et al., 2015) supplied the anatomical substrate of the perivenous outflow that the glymphatic system requires. The meningeal lymphatic genes — *PROX1* (the master transcription factor of lymphatic endothelium), *FLT4* (VEGFR-3, the principal lymphatic growth-factor receptor), *VEGFC* (the principal ligand), *FOXC1* (the forkhead transcription factor whose 6p25.3 locus appears in the WMH GWAS), and the broader lymphatic-development gene set — together specify the genetic architecture of the meningeal-lymphatic drainage component of the glymphatic axis. The age-dependent reduction in meningeal lymphatic capacity (Da Mesquita et al., 2018) is a load-bearing component of the parenchymal solute accumulation that the framework attributes to Phase 0 dysfunction.

10.5 The Glymphatic Genome as a Phase 0 Substrate

The glymphatic gene set — *AQP4*, *DTNA*, *DTNB*, *DAG1*, *SNTA1*, *MLC1*, *GFAP*, *PROX1*, *FLT4*, *VEGFC*, *FOXC1*, *PITX2* — together constitutes the genetic substrate of the parenchymal-clearance architecture whose failure conditions the accumulation of amyloid-β, tau, and metabolic byproducts in the parenchymal compartment across the adult life course. The gene set is anatomically distinct from the tight-junction, pericyte, and transcytosis gene sets — it operates at the astrocyte-endfoot and meningeal-lymphatic interfaces rather than at the brain endothelium — but it is functionally integrated with them through the perivascular drainage pathway that they jointly define. The candidate Phase-0-targeted intervention surface includes sleep-quality interventions (the glymphatic flow is ten-fold elevated during slow-wave sleep), AQP4-modulating pharmacology (under preclinical development), and the broader class of meningeal-lymphatic-protective interventions whose existence the recent identification of the lymphatic vessels has made possible.

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11. Chapter IX — Mapping the Vascular Genome onto the Three-Phase Architecture

11.1 The Phase Architecture of the Cerebrovascular Genome

The cerebrovascular genome, as characterized in Chapters II–VIII, distributes its load-bearing genes across a phase-resolved architecture that maps cleanly onto the three-phase framework of the companion Genetic Architecture dissertation. The mapping is summarized as follows.

Phase 0 (third through fifth decades, cerebrovascular substrate): the tight-junction genes (*CLDN5*, *OCLN*, *TJP1*, *TJP2*, *F11R*, *JAM2*, *JAM3*), the pericyte-endothelial axis (*PDGFB*, *PDGFRB*, *NOTCH3*, *HTRA1*, *FOXF2*, *FOXC1*), the transcytosis and transporter geometry (*MFSD2A*, *SLC2A1*, *LRP1*, *AGER*, *ABCB1*, *ABCG2*), the VCAM-1/BACE2 gateway (*VCAM1*, *BACE2*, *ADAM17*, *ITGA4*, *ITGB1*, *ICAM1*), the APOE-vascular module (*APOE*, *PPIA*, *MMP9*, *TIMP3*), the Mendelian SVD genes (*NOTCH3*, *HTRA1*, *COL4A1*, *COL4A2*, *TREX1*, *CTSA*, *GLA*), and the glymphatic genome (*AQP4*, *DTNA*, *DTNB*, *GFAP*, *MLC1*, *PROX1*, *FLT4*, *VEGFC*).

Phase I (third through fifth decades, locus-coeruleus substrate): the NAD□ axis (*NMNAT2*, *SARM1*, *NAMPT*, *CD38*, *NMRK1*, *NMRK2*, the sirtuin family), the mitochondrial quality-control machinery (*PINK1*, *PRKN*, *OPA1*, *MFN1*, *MFN2*, *DRP1*, *POLG*, *TFAM*), the autophagy-lysosomal apparatus (*TFEB*, *TFE3*, *ATG7*, *BECN1*, *MAP1LC3B*, *OPTN*, *NDP52*, *SQSTM1*), the integrated stress response (*ATF4*, *DDIT3*, *GADD34*, PERK, GCN2, PKR, HRI), and the locus-coeruleus identity program (*TH*, *DBH*, *SLC18A2*, *ADRA2A*).

Phase II (fifth through seventh decades, microglial-and-oligodendrocyte substrate): the microglial GWAS catalog (*TREM2*, *TYROBP*, *CD33*, the MS4A cluster, *PLCG2*, *ABI3*, *INPP5D*), the complement axis (*C1QA/B/C*, *C3*, *C4A*, *C4B*, *CR1*, *ITGAM*), the homeostatic-microglial identity program (*TGFB1*, *SMAD3*, *SALL1*, *MEF2C*, *SPI1*, *P2RY12*, *CX3CR1*), the ferroptosis defense and execution programs (*GPX4*, *FSP1*, *NFE2L2*, *SLC7A11*, *GCLC*, *ACSL4*, *LPCAT3*, *ALOX15*, *ALOX5*), the oligodendroglial identity program (*MBP*, *MOG*, *PLP1*, *MAG*, *OLIG2*), and the lipid-handling axis (*ABCA7*, *ABCA1*, *CLU*, *PLD3*).

Phase III (seventh decade onward, cortical PNN substrate): the perineuronal-net structural program (*ACAN*, *BCAN*, *NCAN*, *VCAN*, *TNR*, *HAPLN1*, *HAPLN4*), the proteoglycan-synthesis program (*CSGALNACT1/2*, *CHST3*, *CHST11*, *HAS1-3*, *B3GAT1*), the matrix-degradation program (*MMP9*, *MMP2*, *MMP3*, *ADAMTS4*, *ADAMTS5*), the matrix-inhibitor program (*TIMP1*, *TIMP2*, *TIMP3*, *RECK*), the parvalbumin-interneuron identity program (*PVALB*, *GAD1*, *GAD2*, *KCNC1-3*, *LHX6*, *NKX2-1*, *ERBB4*, *NRG1*, *GPHN*), and the endocytic-trafficking GWAS axis (*BIN1*, *PICALM*, *SORL1*, *CD2AP*, *EPHA1*).

11.2 The Phase-Spanning Integrative Nodes

Three genes appear in multiple phase-specific lists and constitute the integrative nodes of the four-phase architecture.

APOE is the master integrative node: it load-bears Phase 0 (the PPIA-MMP9-CLDN5 vascular axis characterized in Chapter VI), Phase I (the lipid-handling and bioenergetic-coupling axis characterized in the Genetic Architecture companion), Phase II (the microglial-state and LDAM phenotype), and Phase III (the complement-PNN attack). APOE's effect is therefore the product of its per-phase effects, with the vascular contribution dominant in the fourth through fifth decades, the lipid and microglial contributions dominant in the fifth through seventh,

and the PNN contribution dominant in the seventh and beyond. The multiplicative-across-phases architecture is the formal basis of the APOE4/4 homozygote's near-Mendelian penetrance for late-onset AD that Fortea et al. (2024) characterized.

TREM2 is the second integrative node: its principal effect is in Phase II (the microglial substrate-bridge and the disease-associated-microglia transition), but it also contributes to Phase 0 through its expression on perivascular macrophages and through its modulation of the BBB-resident myeloid compartment, and to Phase III through its complement-modifier effects on PNN integrity. The TREM2 effect is dominant in Phase II but extends to the bridge between Phase 0 and Phase II through the perivascular-macrophage axis.

MMP9 is the third integrative node: it load-bears Phase 0 (the APOE4-driven pericyte-MMP9-CLDN5 axis), is induced as an inflammatory effector in Phase II, and is the proximate executor of Phase III through the matrix-degradation program that consumes the perineuronal net. The *MMP9*-locus inherited variation is therefore a substrate that contributes to disease across the entire phase architecture and supplies a candidate single-target intervention whose effect would be expected to act at multiple phases simultaneously — though with the proviso that the protein's physiological roles in tissue remodeling, leukocyte transmigration, and neural plasticity impose substantial constraints on the therapeutic window.

11.3 The Orthogonality of the Vascular and Parenchymal Genomes

The principal architectural finding of the present chapter is that the Phase 0 vascular genome is largely orthogonal to the Phase I, II, and III parenchymal genomes. The tight-junction genes, the pericyte-endothelial axis, the transcytosis machinery, and the Mendelian SVD genes are not substantially represented in the AD GWAS catalog, and the AD GWAS genes are not substantially represented in the cerebrovascular and stroke GWAS catalogs. The orthogonality is a feature of the architecture rather than a defect of measurement: the vascular and parenchymal substrates are cellularly distinct, are anatomically distributed in different patterns, and respond to different upstream regulators. The orthogonality means that an individual's Phase 0 risk and their Phase I–III risk are inherited as semi-independent components, and that the joint risk profile of an individual is best represented as a multidimensional vector with separate Phase 0, Phase I, Phase II, and Phase III contributions rather than as a single summary score.

The architecture has direct implications for the construction of polygenic risk scores. A flat PRS that sums variant effects across the AD GWAS catalog will systematically misrepresent the Phase 0 contribution of individuals whose vascular substrate is compromised and will fail to identify the individuals at highest risk for the Phase-0-to-Phase-II bridge events (BBB breakdown, VCAM-1-gated microglial recruitment). The phase-weighted PRS architecture proposed in the Genetic Architecture companion should therefore be extended to a four-phase weighted PRS that includes a Phase 0 component derived from the cerebrovascular and SVD GWAS catalogs and from the imaging-genetics literature on WMH burden and BBB permeability.

11.4 The Vascular Integration of the Mendelian Syndromes

The Mendelian neurodegeneration genes — *APP*, *PSEN1*, *PSEN2*, *MAPT*, *GRN*, *C9ORF72*, *SNCA*, *LRRK2*, *GBA*, *TARDBP*, *FUS*, *SOD1*, *HTT* — were treated in the Genetic Architecture companion as principally parenchymal genes whose effector pathways converge on proteostasis, mitochondrial quality control, endolysosomal trafficking, RNA-binding-protein biology, and cytoskeletal-axonal integrity. The vascular integration of these syndromes has been less extensively examined and is a candidate frontier of the present framework. The APP/PSEN-driven autosomal-dominant AD trajectory includes a substantial CAA component, with cerebral amyloid angiopathy contributing to the cognitive presentation and to the ARIA-equivalent imaging findings on antibody therapy. The GBA-driven Parkinson's trajectory includes a vascular component through the cerebrovascular lysosomal-lipid-storage axis. The HTT-driven Huntington trajectory includes a vascular component through the huntingtin protein's role in endothelial trafficking. The C9ORF72 trajectory includes a vascular component through the protein's RAB-GEF function at the endolysosomal-vascular interface. Each of these connections supplies a candidate vascular axis whose elucidation would extend the phase-zero framework into the Mendelian-neurodegeneration core.

12. Chapter X — Therapeutic Implications and Phase-0-Stratified Intervention

12.1 The Phase-0-Targeted Intervention Landscape

The therapeutic landscape of phase-zero intervention is structured by the substrate at which each intervention acts. The tight-junction substrate is addressable by HDAC inhibitors and Wnt-β-catenin pathway modulators that enhance *CLDN5* expression; clinical-stage agents exist in oncology and in neurology and could be repurposed. The pericyte-endothelial axis is addressable by PDGFRβ agonists (in early preclinical exploration), by NOTCH3-targeted antisense oligonucleotides (in development for CADASIL, with potential extension to *NOTCH3*-carrier-stratified late-onset populations), and by the broader class of TGF-β modulators that the *HTRA1* mechanism implicates. The transcytosis substrate is addressable by MFSD2A-targeted DHA-LPC supplementation (now in early clinical trial as a brain-bioavailability strategy) and by RAGE antagonists (azeliragon and analogues, with mixed clinical results). The VCAM-1 gateway is addressable by natalizumab (the approved anti-α4β1 antibody, with substantial multiple-sclerosis safety data, whose AD repurposing has been considered), by anti-VCAM-1 antibodies (under development), and by BACE2-selective inhibitors (whose existence has been forced by the proteomic identification of VCAM-1 as BACE2's principal substrate). The APOE-vascular axis is addressable by cyclophilin A inhibitors (cyclosporine analogues with mechanistic confirmation) and by selective MMP-9 inhibitors. The glymphatic substrate is addressable by sleep-quality interventions, by AQP4 modulators, and by the broader class of meningeal-lymphatic-protective interventions.

12.2 The Genotype-Stratified Anti-Amyloid Antibody Question

The clinical reality of late-onset AD intervention in 2026 is dominated by the approval and increasing use of anti-amyloid monoclonal antibody therapy — lecanemab, donanemab, and to a lesser extent aducanumab — for early symptomatic AD. The disease-modification effect of these antibodies is real but modest, and the ARIA toxicity is substantial and disproportionate in APOE4 homozygotes. The phase-zero framework supplies a direct interpretation of this toxicity profile and a clear stratification principle for intervention.

The interpretation is that ARIA is a Phase 0 event: it is a vascular event that occurs in the vessel wall, not a parenchymal event that occurs in the neuronal cytosol. The mechanism is the destabilization of CAA-affected vessel walls by the amyloid-clearance event that the antibody enables — a mechanism whose magnitude is a function of the patient's CAA burden, which is in turn a function of the patient's lifetime Phase 0 vascular trajectory, which is in turn a function of the patient's vascular genome (principally APOE, with contributions from the heterozygous SVD-gene carriers and the polygenic SVD score). The ARIA risk is therefore *predictable* from the vascular genome, and the stratification principle is direct: the appropriate dose, the appropriate dosing schedule, and the appropriate concomitant therapy (Phase 0 protective intervention concurrent with antibody therapy) are all functions of the patient's Phase 0 risk profile.

The clinical implementation of phase-zero stratification would proceed as follows. The candidate patient for anti-amyloid antibody therapy is assessed at baseline by APOE genotype, by *HTRA1* and *NOTCH3* carrier status (low-cost gene-panel sequencing), by hippocampal BBB permeability on DCE-MRI, by CSF sVCAM-1 and sPDGFRβ, and by baseline cerebral microbleed count on susceptibility-weighted MRI. A patient with low Phase 0 risk (APOE3/3, no SVD-gene carrier status, intact BBB, normal vascular biomarkers, no microbleeds) is dosed at full conventional schedule with standard ARIA monitoring. A patient with intermediate Phase 0 risk is dosed at reduced schedule with intensified monitoring and concurrent Phase 0 protective intervention (cyclosporine analogue at low dose, MFSD2A-DHA-LPC supplementation, blood pressure optimization, sleep-quality intervention). A patient with high Phase 0 risk (APOE4/4, *HTRA1* heterozygous carrier, hippocampal BBB permeability above the Montagne reference range, elevated CSF sVCAM-1, multiple microbleeds at baseline) is considered a candidate for phase-zero-first intervention — Phase 0 protection for two to four years to stabilize the vascular substrate before any antibody-directed parenchymal-clearance therapy is initiated. The framework therefore inverts the conventional ordering, in which parenchymal therapy is initiated first and vascular protection is added if ARIA develops, into a phase-zero-first ordering, in which the vascular substrate is stabilized before the parenchymal substrate is challenged.

12.3 The Mendelian SVD-Carrier Population as a Distinct Trial Population

The Mendelian SVD-carrier population — the *NOTCH3*-cysteine-variant carriers, the *HTRA1*-heterozygous-LOF carriers, the *COL4A1/A2* and *TREX1* heterozygotes, the *GLA* carriers — together comprise a population of approximately one in 200 to one in 500 adults whose Phase 0 trajectory is genetically accelerated and whose late-onset cognitive impairment will likely emerge through a vascular-dominant mechanism. The population is a candidate distinct trial population for phase-zero intervention, with trial endpoints derived from the cerebrovascular biomarker and imaging suite rather than from the parenchymal AT(N) framework. The population is also a candidate population for the early clinical evaluation of phase-zero-targeted interventions because its accelerated trajectory compresses the time to clinical endpoint and increases the statistical power of trials at any given sample size.

12.4 The Lifestyle and Risk-Factor Intervention Surface

The phase-zero framework supplies a direct mechanistic interpretation of the well-established association of midlife vascular risk factors — hypertension, dyslipidemia, type 2 diabetes, hyperhomocysteinemia, chronic systemic inflammation, sleep apnea — with late-life dementia risk. Each of these risk factors operates through the Phase 0 cerebrovascular substrate, and each impinges on the same gene set — *CLDN5*, *PDGFRB*, *MFSD2A*, *AGER*, *LRP1*, *VCAM1* — whose age-dependent reduction the framework treats as the proximate Phase 0 substrate. The lifestyle and risk-factor intervention surface — antihypertensive therapy, statin therapy, GLP-1-receptor agonists, sleep-apnea treatment, exercise, Mediterranean dietary patterns — is therefore the de facto Phase 0 intervention surface that the population has been treating without explicit framework attribution. The phase-zero framework supplies the substrate-level interpretation of why these interventions are protective and the prediction that their effects will be largest in the populations whose Phase 0 substrate is most vulnerable: the APOE4 carriers, the SVD-gene heterozygotes, and the individuals with the highest polygenic Phase 0 risk scores.

12.5 Falsifiable Predictions

The phase-zero framework generates a set of falsifiable predictions that can be evaluated against existing cohort data and against prospective intervention trials.

Prediction 1. CSF sVCAM-1 elevation precedes amyloid PET positivity by five to ten years in cognitively normal adults, with the temporal lead longest in APOE4 carriers and SVD-gene heterozygous carriers. The prediction is testable in the longitudinal cohorts (Framingham, Rotterdam, UK Biobank imaging substudy) that have collected both vascular biomarkers and amyloid imaging.

Prediction 2. ARIA-E and ARIA-H incidence on lecanemab and donanemab therapy are a positive function of baseline CSF sVCAM-1, of baseline CSF sPDGFRβ, and of baseline hippocampal BBB permeability on DCE-MRI, after adjustment for APOE genotype and baseline microbleed count. The prediction is testable in the real-world dosing cohorts that have collected baseline biomarker measurements.

Prediction 3. *HTRA1* heterozygous-LOF carriers and *NOTCH3* cysteine-variant carriers exhibit accelerated CSF p-tau and total tau elevation in the sixth and seventh decades relative to non-carriers matched for age and APOE genotype, with the carrier-effect mediated through the Phase 0 vascular substrate rather than through direct effects on the tau-handling pathway. The prediction is testable in the ADNI and Rotterdam cohorts.

Prediction 4. Pharmacological suppression of the *PPIA-MMP9* axis with cyclosporine analogues (at sub-immunosuppressive doses) or with selective MMP-9 inhibitors in APOE4 homozygotes reduces hippocampal BBB permeability on DCE-MRI, reduces CSF sVCAM-1, and slows the cognitive trajectory in early symptomatic AD. The prediction is testable in a Phase 2 randomized controlled trial design with imaging and CSF endpoints at six and twelve months.

Prediction 5. Sleep-quality interventions (CPAP for sleep apnea, sleep-stage-targeted pharmacology for slow-wave-sleep augmentation) reduce CSF amyloid-β42 accumulation and reduce the slope of cognitive decline in cognitively unimpaired adults with elevated CSF p-tau and intact BBB, with the effect mediated through preservation of glymphatic clearance. The prediction is testable in the existing sleep-intervention cohorts.

13. Conclusion

The genetic architecture of late-onset neurodegeneration is not flat, and it is not parenchymal end-to-end. The architecture is layered, and the first layer is vascular. The cerebrovascular substrate of the brain — endothelial cells, pericytes, vascular smooth muscle, perivascular macrophages, the basement membrane, the astrocytic endfoot, and the meningeal-lymphatic drainage — is itself a genetically encoded cellular system whose inherited variation begins to load-bear in the third and fourth decades and conditions every subsequent phase of the disease trajectory.

This dissertation has developed the case that the vascular dimension is a Phase 0 architecture, that the genes that load-bear Phase 0 are largely orthogonal to the parenchymal genome of Phases I–III but converge on it through three integrative nodes (APOE, TREM2, and the matrix-metalloproteinase family), and that the failure to model Phase 0 as a genetically structured first stage is the principal explanation both for the disappointing performance of stratification-naïve trial designs and for the disproportionate ARIA toxicity of anti-amyloid monoclonal antibody therapy in APOE4 homozygotes.

The framework supplies a four-layer reading of the genetic architecture: Phase 0 (cerebrovascular substrate, third through fifth decades), Phase I (locus-coeruleus substrate, third through fifth decades, partially conditioned by Phase 0), Phase II (hippocampal microglial substrate, fifth through seventh decades, bridged from Phase 0 through the VCAM-1 gateway and from Phase I through the bioenergetic-to-microglial coupling), and Phase III (cortical PNN substrate, seventh decade onward). The four-layer reading replaces the single-number polygenic risk score with a four-dimensional vector, and it replaces the parenchymally-anchored AT(N) biomarker framework with a four-substrate biomarker suite that includes the vascular markers (sVCAM-1, sPDGFRβ, CSF claudin-5, DCE-MRI BBB permeability) alongside the AT(N) parenchymal markers.

The clinical translation of the framework is direct. Trials of disease-modifying therapy should stratify on Phase 0 vascular biomarkers and vascular genotype, not only on parenchymal AT(N) status. Anti-amyloid antibody therapy should be dosed and monitored as a function of the patient's Phase 0 risk profile, with consideration of phase-zero-first intervention in the highest-risk patients. The Mendelian SVD-carrier population is a distinct trial population whose accelerated trajectory

makes them informative for the early clinical evaluation of phase-zero-targeted interventions. The lifestyle and risk-factor intervention surface that the population has been treating without explicit framework attribution is, in fact, the de facto Phase 0 intervention surface, and the phase-zero framework supplies the substrate-level interpretation of why these interventions work.

The deeper claim of the present dissertation is that the cerebrovascular substrate is not a comorbidity. It is the first phase of the disease, it has a genome, and the genome can be read. The vascular dimension belongs in the first principles of late-onset neurodegeneration, not in the appendix.

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14. References

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End of dissertation.