THE ARCHITECT'S UNDERSTUDY

Can a Drug Stand In for the Reelin-COLBOS Variant? — Why the Literal Phenocopy Is Foreclosed, Why the Functional Phenocopy Is Not, and What the Last Fourteen Residues of Reelin Reveal About Where a Drug Must Aim

The Design Brief · The Last Fourteen Residues · Four Locked Doors
Five Open Ones · The Knife-Edge · The Understudy

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The pharmacology volume of the reelin series. Where The Architect's Reprieve, Scaffold and Handshake traced the brake, the matrix that stages it and the three-body clasp that fires it, this one asks the translational question they set up and did not answer: given a man who resisted a deterministic dementia for twenty-three years on one substitution in reelin, what molecule would do the same for someone who lacks it?

"Ordinarily we must guess whether correcting a target would help, and the history of this disease is largely a history of guessing wrong. Once in a generation a person is born who has already run the experiment — one substitution, one lifetime, an unambiguous readout. Then the question is no longer whether the correction works. It is only whether we can make one."

— ONS Methodology Working Notes, 2026

For the man from Antioquia who carried a mutation that grants no reprieve to anyone else, and was given twenty-three years by fourteen residues at the end of a protein — and for his sister, who was given twelve.

THE REELIN SERIES THE PHARMACOLOGY VOLUME

The Architect's Reprieve (reelin as the brake on tau; the rising-reelin paradox)

The Architect's Scaffold (the sulfated matrix that stages the brake)

The Architect's Handshake (the three-body clasp: reelin — N-sulfated HS — ApoER2)

The Architecture of Resistance (the atlas of protective alleles this variant belongs to)

The Architect's Understudy ← this volume

The three preceding reelin volumes are volumes of mechanism. They establish that reelin brakes tau, that the brake must be staged on a sulfated surface, and that the signal fires only when ligand, sugar and receptor close a three-body clasp together. Each ends at the same threshold and declines to cross it. This volume crosses it. It takes the one human being in whom that clasp demonstrably worked better than it does in the rest of us — a heterozygous carrier of RELN-COLBOS who resisted a fully penetrant dementia for twenty-three years — and asks what it would take to build a drug that does the same. The answer begins with a fact the field has passed over: the variant does not improve reelin's grip on its receptor at all. It improves the staging. Which means the molecule that best imitates it is one that contains no reelin whatever.

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Abstract

In 2023 a man from the Antioquia kindred of Colombia was described who carried the fully penetrant *PSEN1*-E280A mutation — a genotype that produces mild cognitive impairment at a kindred median of forty-four years — and who remained cognitively intact until sixty-seven. He was not a carrier of the Christchurch variant of apolipoprotein E, the only protective factor then known in that kindred. He carried, heterozygously, a single substitution in the reelin gene: histidine to arginine at residue 3447, named COLBOS for the Colombia–Boston study that found it. His amyloid burden was extreme; his entorhinal tau signal was strikingly limited; and a knock-in mouse bearing the murine equivalent of his variant showed stronger activation of reelin's canonical adaptor Disabled-1 and lower phosphorylation of human tau. This dissertation asks the question that case makes unavoidable and that no published work has yet asked systematically: **could a drug do what that variant did?**

The answer turns on a fact that is easy to state and consequential enough to reorganize the entire design problem. Residue 3447 is not in the part of reelin that binds reelin's receptors. The receptor-binding activity of the protein resides entirely in a central fragment spanning repeats three through six, at residues 1220–2664, and within that fragment in a two-repeat module whose double-lysine grip on the first ligand-binding module of ApoER2 has been resolved crystallographically. Residue 3447 lies seven hundred and eighty-odd residues beyond the end of that fragment, fourteen residues from the protein's carboxy terminus, inside a thirty-two-residue basic tail — the C-terminal region — whose structure was solved by the COLBOS discovery group themselves. And the discovery paper reports, in a result whose importance has been almost entirely overlooked, that **the COLBOS substitution does not alter reelin's binding to either ApoER2 or VLDLR**. Whatever the variant does, it does not make a better key.

What it does instead is now traceable. The C-terminal region is dispensable for reelin's secretion and dispensable for binding the isolated receptor ectodomain, but required for binding receptor-bearing *membranes* and for efficient downstream signalling — a discrepancy whose authors, in 2007, attributed to an unidentified co-receptor. Two co-receptors have since been named, and COLBOS improves reelin's grip on both: N-sulfated heparan sulfate, for which the variant's affinity rises from seventeen to ten nanomolar, and neuropilin-1, for which it rises roughly tenfold. Reelin signals not by the strength of one contact but by the simultaneity of many; a monomeric receptor-binding fragment is nearly inert and its artificial oligomerization increases activity tenfold; and the co-receptors are what concentrate and align ligand and receptor so that many modest grips close together. **COLBOS is therefore not a better ligand but a better-staged one — a gain in avidity, not in affinity; in residency, not in recognition.** This dissertation takes that as its design brief.

From the brief, four doors close and five open. The literal phenocopy — put the variant, or the variant protein, into a patient — is foreclosed on four independent grounds, any one of which

would suffice. The protein is 388 kilodaltons and no blood-brain-barrier shuttle has ever moved a payload above antibody scale. The gene is 10,383 base pairs of coding sequence, 2.2 times the packaging limit of adeno-associated virus, and oversized genomes are truncated during packaging rather than merely packaged inefficiently. The one reelin construct anyone has ever put into a viral vector is the central fragment — which, by the geometry above, *cannot carry the mutation*. And while the substitution is a textbook adenine-base-editor target at the codon level, no in-vivo central-nervous-system base editing exists in humans; the celebrated 2025 personalized editing case was hepatic, for reasons of lipid-nanoparticle tropism that do not generalize to neurons.

The functional phenocopy is a different matter, and the argument of this dissertation is that it is genuinely viable. If the variant's gain is avidity at the membrane, then a drug need not deliver reelin at all; it need only make the clustering happen. That reframing surfaces a precedent hiding in plain sight since 2004: bivalent agents directed at the ligand-binding domains of ApoER2 or VLDLR — a dimeric receptor-associated-protein fusion, and polyclonal antibodies against each receptor's ligand-binding domain — induce Disabled-1 tyrosine phosphorylation at the same sites reelin uses, activate Src-family kinases, and increase long-term potentiation in hippocampal slices. Clustering is sufficient. No co-receptor is required, because the antibody *is* the clustering agent. **The drug that best phenocopies COLBOS is the one that least resembles reelin.** We rank five open routes — clustering agonism, splice-switching correction of the ApoER2 cytoplasmic exon whose balance is deranged in human Alzheimer's brain, inhibition of the reelin-inactivating protease ADAMTS-3, rescue of ApoE4-induced receptor-recycling blockade, and, last and least tractable, tuning the sulfation of the sugar bed — grading each on mechanistic proximity to the variant, precedent, and liability.

The dissertation then names a collision that neither of the two literatures involved has acknowledged. Pathological tau enters neurons through heparan sulfate proteoglycans, and the field's principal anti-tau tools against that route — heparin, heparinase, knockout of the N-deacetylase/N-sulfotransferase NDST1 — are, term for term, the same three perturbations shown to abolish reelin-induced ApoER2 dimerization. The sugar that admits tau is the sugar that stages the brake on tau. Worse, the resilience variant we propose to imitate binds that sugar *harder* than wild-type: an HS-directed anti-tau drug would not merely risk collateral damage to reelin, it would act directly against the COLBOS mechanism. We verified by full-text inspection that neither literature cites the other. We name this the knife-edge, and we draw from it the design rule that resolves it: **target the protein that reads the sugar, never the sugar itself** — precisely the architecture of the Christchurch-mimetic antibody that the mirror program has already validated in vivo.

We close on an honest ledger. The human evidence is one man, a partially protected sister, and a small oldest-old case series in a low-profile venue. The mouse effects were male-only and required homozygosity where the man was heterozygous. Excessive reelin signalling has its own developmental pathology, so the therapeutic goal is a fine adjustment and not a maximal one — a reading the co-receptor biophysics independently supports, since the variant's affinity gain is

roughly twofold and its own discoverers call it a fine-tuning. Chronic pathway agonism is self-limiting, because reelin signalling triggers the ubiquitin-mediated destruction of the very adaptor it phosphorylates. And the kinase downstream is one the field has already tried and failed to inhibit directly. None of this is disqualifying; all of it is design constraint. The verdict is that a drug cannot copy this variant, and does not need to. An understudy is not a double. It does not have to be the same actor; it has to hit the same marks.

I. The Question, Put Precisely

There is a particular kind of question that human genetics occasionally hands to pharmacology, and it is worth being clear about its structure before attempting to answer it. Ordinarily a drug programme begins with a target nominated by disease biology — a protein that is too abundant, or too active, or misfolded — and proceeds by asking what molecule might correct it. The evidence that the correction will help is inferential, assembled from models, and the history of Alzheimer's disease therapeutics is in large part a history of that inference failing. Occasionally, however, the order is reversed. Occasionally nature performs the experiment first, in a human being, over a lifetime, with a fixed genetic perturbation and an unambiguous clinical readout. When that happens the question is no longer *would correcting this help?* but the far more tractable *can we reproduce, pharmacologically, a correction we already know helps?*

The Antioquia kindred has furnished two such experiments. Both concern carriers of *PSEN1*-E280A, a mutation of complete penetrance that produces mild cognitive impairment at a kindred median of forty-four years and dementia at forty-nine. Both concern individuals who deviated from that schedule by roughly three decades. The first, described in 2019, was a woman homozygous for the Christchurch variant of apolipoprotein E, arginine-136 to serine, who reached her seventies with a very high amyloid burden but limited tau pathology and limited neurodegeneration.[1] The second, described in 2023, is the subject of this dissertation. He was a man, heterozygous for a variant of reelin — histidine 3447 to arginine, named COLBOS — cognitively intact until sixty-seven, mildly impaired at seventy, demented at seventy-two, dead at seventy-four; and at autopsy his brain met every criterion for severe Alzheimer's disease, CERAD C, Braak VI, Thal phase 5, weighing seven hundred and forty-five grams.[2] He was, by neuropathology, as sick as anyone in the kindred. By clinical course he had been given twenty-three years.

The imaging is where the mechanism first shows itself. His cortical amyloid, measured by Pittsburgh compound B at seventy-three, ran to a distribution volume ratio of 1.77 — higher than the 1.51 average of younger, already-impaired carriers. His tau, measured by flortaucipir, was ordinary in inferior temporal cortex but conspicuously limited in the entorhinal cortex, at a standardized uptake value ratio of 1.34, and limited in posterior cingulate and precuneus. His investigators single out the entorhinal finding as "a salient feature… that could be critical for the protection phenotype," and note that the Christchurch case shares it.[2] This is the signature that organizes everything that follows. **Amyloid was not prevented. Tau was.** And it was prevented at the anatomical station where the disease ordinarily takes hold.

The genetics were followed with mechanism. The COLBOS substitution was shown to increase phosphorylation of Disabled-1 — reelin's canonical intracellular adaptor — in primary cortical neurons relative to wild-type reelin. A knock-in mouse carrying the murine equivalent, H3448R, showed enhanced Disabled-1 phosphorylation in cerebellum, and when crossed onto the P301L human-tau line reduced hippocampal and medullary phospho-tau at the Thr205 epitope and rescued the limb-clasping phenotype.[2] A gain-of-function in reelin, a stronger signal through Disabled-1, less phosphorylated tau, and three decades of cognition against a mutation that grants no reprieve to anyone else: the causal chain is as complete as the ethics of the disease will ever permit.

So the question of this dissertation is well-posed, and it is not rhetorical. Can a drug do that? Not *could reelin biology be therapeutically interesting* — a question the field has circled for two decades without resolution — but the specific and answerable version: given what this variant does, at this residue, in this protein, what molecule or modality would reproduce its effect in a patient who does not carry it, and how far is that molecule from existing?

The answer requires three things in order, and this dissertation supplies them in that order. First, an exact account of what the variant does — which turns out to be materially different from what the shorthand "gain-of-function reelin" implies, and different in a way that changes the entire design problem. Second, a design brief derived from that account: a statement of what a drug would actually have to reproduce, and what it would not. Third, a survey of the modalities, honest about which are foreclosed and which are open, ranked by mechanistic proximity to the variant rather than by fashion.

We begin, as any such programme must, by reading the residue.

II. What the Variant Actually Does

The Man, the Sister, and the Cohort

Before the molecule, the phenotype, and its limits. The proband is one person. That is the first and most important thing to say about the human evidence, and no amount of mechanistic corroboration downstream changes it. But he is not quite alone.

His sister carried the same two variants — *PSEN1*-E280A and RELN-COLBOS — and she was protected too, though less: mild cognitive impairment fourteen years late, dementia twelve years late, with onset at sixty-one and death at seventy-three.[2] Her investigators record the confounds honestly: severe head injury, depression, hypothyroidism. Two carriers, both protected, one substantially more than the other. A dose–response of sorts, and also a warning that the variant sets a probability rather than guaranteeing an outcome.

Beyond the kindred, a 2025 case series identified five RELN-COLBOS carriers among a hundred and thirty-five Colombian oldest-old individuals, including a hundred-and-two-year-old with preserved cognition and relatively preserved cortical glucose metabolism.[3] The finding is suggestive and is consistent with a protective allele enriched among survivors. It also appeared in a non-standard venue, is descriptive, and is uncontrolled, and this dissertation grades it accordingly — as supporting colour, not as evidence of effect. The honest state of the human data is: one well-characterized protected man with autopsy, one less-protected sibling, and a handful of carriers who lived a long time.

The Residue and Its Neighbourhood

Human reelin is three thousand four hundred and sixty amino acids. Its architecture, after a signal peptide and an F-spondin-like region, is eight tandem "reelin repeats," each an unusual bipartite fold split by an EGF-like module, and then — after the last repeat ends near residue 3373 — a short, highly basic tail of thirty-two residues known as the C-terminal region.[4] Residue 3447 sits inside that tail, fourteen residues from the end of the protein.

This is the fact from which everything in this dissertation follows, and it deserves to be stated against the alternative that most readers will assume. The receptor-binding activity of reelin does not reside in the C-terminal region. It resides, entirely, in a central fragment generated by proteolysis in vivo, spanning repeats three through six at residues 1220 to 2664, which is both necessary and sufficient to bind the receptors, to phosphorylate Disabled-1, and to rescue the disordered cortex of the reeler mouse in slice culture.[5] Within that fragment the activity narrows

further, to the module formed by the fifth and sixth repeats, whose two-ångström crystal structure identified lysine-2360 and lysine-2467 as the centre of the receptor contact,[6] and whose complex with the first ligand-binding module of ApoER2 was later resolved at 2.6 ångströms, showing the "double-lysine" recognition mode shared across the endocytic lipoprotein receptors.[7]

Residue 3447 lies seven hundred and eighty-three residues past the end of the central fragment. It is not in the receptor-binding module. It is not in the receptor-binding fragment. It is in a tail that the receptor-binding literature has, for most of two decades, treated as an appendage.

The COLBOS discovery group evidently did not treat it that way. They solved the structure of the thirty-two-residue C-terminal region and deposited it in the Protein Data Bank alongside the clinical report. They located the action in the tail because that is where the biology told them to look.

The Negative Result That Organizes Everything

Buried in the 2023 paper, and to our reading almost entirely unremarked in the subsequent discussion of it, is a cell-free binding experiment with a null result. **The COLBOS substitution did not alter reelin's binding to VLDLR, and did not alter its binding to ApoER2.**[2]

Take that seriously and the popular shorthand collapses. "Gain-of-function reelin" invites the picture of a ligand that grips its receptor more tightly and so signals more strongly — the picture under which a drug would be a better-binding reelin analogue, or a small molecule occupying the reelin site, or an engineered fragment with improved affinity. That picture is wrong. The variant's advantage is not at the receptor interface at all. Whatever three decades of cognition were bought with, they were not bought with tighter receptor binding.

And this is fortunate, because the receptor interface would have been a miserable target. The reelin–LA1 contact buries only about three hundred and fifty square ångströms per face — a small, calcium-dependent, deliberately reversible grip.[7] Interfaces of that size are notoriously poor substrates for small-molecule agonism; there is not enough surface to build against. Had COLBOS worked by improving that contact, the design problem would have been close to hopeless. It works somewhere else.

What Did Change: The Co-Receptors

The C-terminal region's function was characterized, with unusual prescience, sixteen years before the COLBOS variant was found. Deleting or replacing the region does not impair reelin's secretion — correcting an earlier inference from the Orleans reeler allele — and does not impair binding to the isolated receptor *ectodomain*. But C-terminal-region-less reelin binds isolated cell membranes and receptor-expressing neurons far more weakly, and signals far less potently. The authors concluded that the region works "presumably via binding to an unidentified 'co-receptor' molecule(s) on the cell membrane."[8] A knock-in mouse lacking the region later confirmed the functional consequence in vivo: Disabled-1 protein accumulates, the signature of weakened signalling, and postnatal cortical dendrites are misoriented and poorly branched.[9]

Two co-receptors have since filled that blank, and the COLBOS variant improves reelin's grip on both.

The first is neuropilin-1, which binds C-terminal-region-intact reelin, forms a complex with VLDLR, and augments signalling — and whose entire contribution is abolished by proteolytic removal of the last six residues of reelin, 0.17 per cent of the protein.[10] The C-terminal-region peptide bearing the COLBOS substitution shows roughly tenfold higher affinity for neuropilin-1 than wild-type.[2]

The second, and better characterized, is heparan sulfate. A 2025 study established that full-length reelin binds heparan sulfate with high affinity; that the determinant is N-sulfation specifically, since N-desulfated heparin loses binding while 2-O- and 6-O-desulfation barely matter; that heparinase treatment or knockout of the N-deacetylase/N-sulfotransferase NDST1 reduces reelin surface binding to roughly a third; and — the functional claim — that heparinase or soluble heparin markedly reduces reelin-induced ApoER2 dimerization in a split-luciferase assay, while N-desulfated heparin does not.[11] The same study measured the variant directly:

Construct	Affinity for heparan sulfate (K_D)
Full-length reelin, wild-type	17 ± 5 nM
Full-length reelin, COLBOS	**10 ± 2 nM**
C-terminal region, wild-type	65 ± 23 nM
C-terminal region, COLBOS	**32 ± 9 nM**

Two things in that table deserve emphasis. First, the isolated thirty-two-residue tail binds with affinity on the same order as the entire four-hundred-kilodalton protein — the tail *is* the heparan-sulfate-binding site of reelin. Second, the COLBOS gain is modest. The authors are explicit and careful about this: the variant "shows a modest (~1.5–2-fold) increase in overall affinity

and a subtle shift in glycan preference," and it "fine-tunes, rather than dramatically increases, Reelin–HS interactions."[11] The substitution does not alter the recognition mechanism. It nudges it.

The structural bookkeeping is finer still. The discovery paper maps two glycosaminoglycan-binding determinants within the tail: an α-site in the last six residues, overlapping the neuropilin-1 site and removable by furin cleavage, and a β-site at residues 3446–3451.[2] Residue 3447 sits inside the β-site. The variant is a single arginine substituted into a sugar-binding patch of a basic tail — which is, chemically, exactly what one would design if one wanted a marginally better grip on a polyanion.

Reading the Gain: Avidity, Not Affinity

We can now state what the variant does in a form a drug programme can use.

Reelin does not signal by the strength of a single contact. The receptor-binding module is engineered for the opposite: a small, reversible, calcium-dependent grip.[7] Potency comes instead from simultaneity — from many modest grips closing at once across an array of clustered receptors. The demonstration is direct and quantitative: a monomeric receptor-binding fragment is nearly inert, and forcing it into oligomers raises its activity roughly tenfold.[6] A parallel result makes the same point from the other direction. Of reelin's hundred and twenty-two cysteines, one — Cys2101 — mediates covalent homodimerization; the C2101A mutant still binds ApoER2 and VLDLR normally by surface plasmon resonance, and still fails to signal in neurons.[12] Receptor affinity is preserved; higher-order architecture is broken; signalling is lost. Affinity is not the potency variable. Architecture is.

Set the COLBOS data against that frame and the reading is immediate. The variant leaves receptor affinity untouched and improves the interactions that govern where reelin sits and how long it stays there: a twofold tighter grip on the N-sulfated sugar bed that concentrates and aligns it, a tenfold tighter grip on the transmembrane co-receptor that partners VLDLR. It does not make the handshake stronger. **It makes the handshake more likely to happen, and to happen in company.** The gain is in residency and avidity — in the probability that, on a given patch of dendritic membrane, enough grips close together for the signal to fire.

That is the design brief, and it is a considerably better one than the alternative. A drug asked to improve a three-hundred-and-fifty-square-ångström protein–protein contact has almost nowhere to bind. A drug asked to increase the local clustering of a receptor has a great many places to stand.

III. The Design Brief

It is worth writing the brief down explicitly, in the form a development team would use, because doing so immediately disqualifies several intuitively appealing approaches and licenses several counterintuitive ones.

Requirement	What the variant does	What a drug must therefore do	What a drug need *not* do
Primary mechanism	Raises avidity of reelin at the neuronal membrane via co-receptor engagement	Increase the frequency of ApoER2/VLDLR clustering events	Improve reelin's receptor affinity — the variant does not
Proximate readout	Increased Disabled-1 tyrosine phosphorylation	Produce Disabled-1 phosphorylation at the reelin-specific sites	Deliver reelin protein, or any reelin-derived molecule
Distal readout	Reduced tau phosphorylation; entorhinal tau limited	Lower phospho-tau, with anatomical priority on entorhinal cortex	Reduce amyloid — the proband's amyloid was extreme
Magnitude	~1.5–2× co-receptor affinity; "fine-tunes rather than dramatically increases"	Aim for a modest, sustained increase in pathway tone	Maximize pathway activation — overdrive has its own pathology
Duration	Germline; present from conception, acting across a lifetime	Be tolerable for years to decades	Act acutely; there is no acute readout to chase
Timing	Protection was against *onset*, over decades	Target prodromal or presymptomatic populations	Rescue established dementia
Zygosity	Heterozygous in the protected man	Achieve a partial, one-allele-equivalent effect	Achieve full pathway saturation

Four entries in the right-hand column are worth dwelling on, because each closes a line of enquiry that a less careful reading would leave open.

A drug need not deliver reelin. This is the liberating consequence of the negative binding result. If the variant's advantage were an improved ligand, then the ligand would be the product, and the programme would be a protein-delivery programme with all of that discipline's constraints. It is not. The advantage is in the staging of an interaction that endogenous reelin already participates in. The

endogenous ligand is present — in the Alzheimer's brain, as we shall see, it is more than present — and the therapeutic question is whether its existing engagement can be made more productive.

A drug need not reduce amyloid. The proband's cortical amyloid exceeded that of impaired carriers thirty years his junior. Whatever reelin's relationship to amyloid may be in transgenic mice — and overexpression does delay fibril formation in one well-known model[13] — the human protection here ran through tau, on a background of unchecked amyloid. A programme that measured itself against amyloid endpoints would be measuring the wrong thing, and would probably fail.

A drug should not maximize the pathway. The affinity gain is roughly twofold, and the paper reporting it says so in those words. The discovery paper adds that the hypermorphic effect is mild and speculates that a stronger one "may not support proper development." The biology corroborates: mice in which the reelin-inactivating protease ADAMTS-3 is deleted, or in which reelin is rendered cleavage-resistant, show developmental abnormalities of their own from excessive signalling.[14] This is a rheostat with a narrow useful range, not a switch to be thrown.

A drug should target early disease. The variant delayed onset by twenty-three years in a man whose brain nevertheless accumulated Braak VI pathology. It bought time before the clinical threshold, not recovery after it. The corresponding trial is a prevention or prodromal trial, with the enrolment, duration and endpoint problems that implies, and no honest programme should pretend otherwise.

IV. Four Locked Doors

The literal phenocopy — supply the variant, or the variant's protein, to a brain that lacks it — is the first thing anyone proposes and the first thing that has to be abandoned. It fails on four independent grounds. Any one of them alone would be a programme-ending obstacle; together they are dispositive, and it is worth walking each because the reasons they fail are what point toward what does not.

Door One: The Protein Is Too Large, and Too Wrong in Shape

Full-length human reelin is 388 kilodaltons of polypeptide before glycosylation, and runs at an apparent 420 to 450 kilodaltons glycosylated.[5] The central fragment is 162 kilodaltons unglycosylated. For comparison, the payload that current blood-brain-barrier shuttle technology has been engineered to move is an immunoglobulin, at roughly 150.

That technology is real and it works, within its limits. Reducing rather than increasing the affinity of an anti-transferrin-receptor arm increases brain uptake, and a bispecific anti-transferrin-receptor/BACE1 antibody lowered brain amyloid after a single systemic dose.[15] High-affinity anti-transferrin-receptor binding instead routes the receptor to lysosomal degradation and reduces brain exposure on subsequent doses — a liability that had to be engineered around.[16] The platform translates to non-human primates.[17] And the best current numbers come from a clinical-stage shuttled antibody whose brain partition coefficient is about 0.5 per cent, seven-fold higher than its unshuttled parent in cerebellum and thirty-three-fold higher in striatum.[18]

Note what those figures mean. Half a per cent, for a payload the platform was designed around, achieved only after two decades of engineering. Nothing above antibody scale has been shuttled. Reelin is two and a half times that mass, is not a compact globular immunoglobulin but an elongated multidomain glycoprotein, and — the point that makes delivery insufficient even if it were achieved — signals by clustering, which requires the multimeric species.[6,12] Delivering monomers of a protein whose potency depends on oligomeric architecture would not obviously reproduce the signal even at the receptor.

Reelin *has* been delivered to rodent brain, and the results are genuinely encouraging within their frame. A single bilateral intraventricular injection of purified full-length reelin into wild-type mice increased Disabled-1 phosphorylation within fifteen minutes, raised dendritic spine density, enhanced CA1 long-term potentiation, and improved associative and spatial memory.[19] The same manoeuvre recovered sensorimotor gating, synaptic plasticity and associative learning in heterozygous reeler mice,[20] and recovered synaptic function and cognition in an Angelman

syndrome model.[21] These are important proofs that the pathway is drivable in an adult brain. They are not proofs of a delivery route: the route was a cannula through the skull into the ventricle, and the species was a mouse.

Door Two: The Gene Does Not Fit in the Vector

The coding sequence of *RELN* is 10,383 base pairs. The packaging limit of adeno-associated virus is approximately 4.7 kilobases. The gene is 2.2 times the vector.

This is not a difficulty to be optimized. Oversized genomes are *truncated during packaging*, with few recovered genomes exceeding five kilobases regardless of input size and yields falling roughly tenfold.[22] A dual-vector split-intein strategy would require each half to carry above five kilobases before promoter, polyadenylation signal and inverted terminal repeats — so a triple split would be needed, and triple-vector reconstitution efficiency is poor. Even the central fragment, at 4,335 base pairs of coding sequence, exceeds the limit once a minimal promoter, a polyadenylation signal and a signal peptide are added.

The rest of the central-nervous-system gene therapy field is in better shape than this constraint suggests, which makes the constraint sharper rather than softer. There is an approved systemically dosed AAV9 product for spinal muscular atrophy.[23] Capsid engineering has moved beyond the notorious AAV-PHP.eB, whose brain tropism turned out to depend on the murine LY6A receptor and therefore does not translate,[24] to variants with brain-wide expression that extend to marmoset,[25] and to a capsid reprogrammed to bind human transferrin receptor 1 that achieves forty- to fifty-fold higher central-nervous-system expression than AAV9 in humanized mice.[26] The delivery problem is being solved. The cargo problem, for this particular cargo, is arithmetic.

Door Three: The Fragment Cannot Carry the Mutation

This is the door that closes most decisively, and it is the one that only becomes visible once the residue has been located properly.

The field's standing workaround for reelin's size is the central fragment: necessary and sufficient for receptor binding and Disabled-1 phosphorylation,[5] and a third the mass of the parent. It is the construct one would vector, and it is the construct that has in fact been vectored — an AAV9 encoding a secreted, bioactive R3–6 fragment, delivered intraventricularly into an amyloid-plus-tauopathy mouse, improved radial-arm-water-maze performance and reduced insoluble Aβ42, though it did not reduce tau pathology. That work is a conference abstract without peer review and is graded here accordingly.

But the central fragment ends at residue 2664. Residue 3447 is not in it. **The one reelin construct small enough to vector is, by geometry, incapable of carrying the COLBOS substitution.** One cannot make a COLBOS central fragment; the phrase is a contradiction. And

the fragment that lacks the C-terminal region is precisely the species shown to bind cell membranes weakly and signal inefficiently[8] — that is, the species lacking exactly the property the variant improves. A vectored central fragment is not a low-dose COLBOS. It is a construct engineered to omit the mechanism.

Door Four: The Edit Is the Right Class and the Wrong Organ

At the codon level the substitution is almost invitingly tractable. Codon 3447 in the reference transcript is CAT, encoding histidine; CGT encodes arginine; the change is a single adenine-to-guanine transition at the middle position, which is the canonical substrate class for adenine base editors. Whether a base editor could actually make this edit depends on protospacer-adjacent-motif availability in that window, on editing-window positioning, and on bystander adenines in the neighbouring codons — none of which we have assessed, and none of which should be assumed.

The organ is the obstacle. In-vivo personalized base editing in a human being is now real: a neonate with severe carbamoyl-phosphate synthetase 1 deficiency received a customized lipid-nanoparticle-delivered base-editing therapy in two infusions and showed increased dietary protein tolerance without serious adverse events.[27] It is a genuine landmark. It is also a *hepatic* landmark, and the reason is mechanistic rather than incidental: lipid nanoparticles distribute to liver through apolipoprotein-E-mediated uptake, which is why a urea-cycle enzyme was the achievable first target. There is no in-vivo central-nervous-system base editing in humans, and none we could verify in primates. A *RELN* edit would additionally require a rationale for why editing one allele in some fraction of post-mitotic neurons in late life reproduces a germline heterozygous gain-of-function present from conception — a question the developmental biology of reelin makes non-trivial.

The Four Doors, Read Together

Route	The obstacle	Severity
Deliver reelin protein	388 kDa; nothing above IgG scale has been shuttled; potency requires multimers	Foreclosed for systemic delivery
Deliver the *RELN* gene	10,383 bp coding vs ~4.7 kb limit; oversized genomes truncate	Foreclosed
Deliver a COLBOS fragment	Residue 3447 lies outside the only vectorable fragment	Logically impossible
Edit the residue in vivo	Correct editor class; no CNS delivery exists; germline-vs-somatic mismatch	Foreclosed near-term

Four doors, four different reasons. The pattern is instructive: every one of them is a consequence of trying to reproduce the *molecule*. None of them is a consequence of the *mechanism* being unreachable. That distinction is the turn on which the rest of this dissertation depends.

V. The Turn: Phenocopy the Function, Not the Molecule

Return to the design brief. The variant does not improve reelin's grip on its receptor; it improves the probability that reelin's grip is made in the clustered geometry that fires the signal. Its instrument for doing so is a pair of co-receptors that concentrate ligand and receptor on the same patch of membrane.

Now ask a question the literal framing never permits. If the therapeutic quantity is *receptor clustering*, why route the intervention through the ligand at all? The co-receptors exist because reelin, a soluble protein of modest single-site affinity, needs help achieving multivalency. A drug is not under that constraint. A drug can be built multivalent from the outset.

The answer to this question was published in 2004 and has been sitting in the literature, largely unexploited, ever since. Bivalent agents directed at the ligand-binding domains of the reelin receptors are *sufficient* to reproduce the reelin signal: they induce Disabled-1 tyrosine phosphorylation at the same sites, activate Src-family kinases, modulate Akt, and increase long-term potentiation in hippocampal slices.[28] The agents were three — a receptor-associated protein fused to human immunoglobulin Fc, therefore bivalent for both receptors; a polyclonal antibody against the ApoER2 ligand-binding domain; and a polyclonal antibody against the VLDLR ligand-binding domain. The antibodies induced Disabled-1 phosphorylation "in a manner similar to that of Reelin," dose-dependently. The Fc fusion perfused onto hippocampal slices gave 187 per cent potentiation at sixty minutes post-tetanus against 145 per cent for the Fc control. The study's own title states the conclusion: receptor clustering is involved in reelin signalling.

Read that result against the COLBOS mechanism and the implication is sharp. The variant achieves better clustering *indirectly*, by improving reelin's residency on co-receptors that organize the membrane. A bivalent antibody achieves clustering *directly*, and needs no co-receptor, no sugar bed, no neuropilin, and no reelin. It does in one step what the variant does in three.

The drug that best phenocopies the COLBOS variant is therefore the drug that least resembles reelin.

This is not a rhetorical flourish; it is the practical conclusion of the anatomy. And it converts the programme from a protein-delivery problem, which is foreclosed, to an antibody-engineering problem, which is merely hard — and which the mirror programme in this same kindred has already shown to be executable, as Section IX will describe.

Four further routes follow the same logic of acting on the pathway rather than supplying its ligand, and we take all five in order of mechanistic proximity to the variant.

VI. Five Open Doors

Route One — Clustering Agonism at ApoER2/VLDLR

Mechanistic proximity to the variant: highest.

The proposal is an engineered agonist antibody, or antibody-like multivalent scaffold, directed at the ligand-binding domain of ApoER2 — with VLDLR as an alternative or a co-target — whose therapeutic action is to cluster the receptor and fire Disabled-1.

The precedent is direct: polyclonal antibodies against each receptor's ligand-binding domain already do this, with a functional electrophysiological readout.[28] What has never been done is the engineering — affinity maturation, epitope selection, valency optimization, isotype and Fc design, blood-brain-barrier delivery, and chronic tolerability. That gap is precisely what makes it a programme rather than a result.

The adjacent art is rich enough to derisk the biology substantially. Agonist antibodies to neurotrophin receptors work in the central nervous system: the TrkB agonist 29D7 promotes retinal ganglion cell survival and neurite growth,[29] and given intraventricularly raises phospho-ERK and phospho-Akt, blocks caspase-3 activation, and produces durable neuroprotection and sensorimotor recovery after neonatal hypoxia-ischaemia.[30] The valency engineering problem has been solved repeatedly in oncology, where dual-epitope antibodies with hexamerization-promoting Fc mutations achieve potent agonism independent of Fcγ-receptor crosslinking,[31] and where the structural basis of higher-order receptor clustering was worked out well enough to rescue a failed clinical programme.[32] The field also learned a caution worth importing: some receptors sit in autoinhibitory preligand clusters, so that true agonism requires *converting* a cluster rather than merely aggregating receptors.[33] Whether ApoER2 has such a resting state is unknown and is a first-order question for this route.

There is one structural encouragement specific to this receptor. The full-length ApoER2 ectodomain bound to signalling-competent reelin adopts an intermediate "contracted-open" conformation, and the study that resolved it identified an auxiliary low-affinity interface whose pH sensitivity governs ligand release.[34] A receptor with a defined signalling-competent conformation and a secondary interface is a receptor with more than one place for an engineered agonist to act.

Liabilities. Blood-brain-barrier delivery remains the binding constraint, though at antibody scale it is a solved-in-principle problem with quantified performance.[18] ApoER2 and VLDLR are expressed peripherally and have lipoprotein-metabolic functions; chronic systemic agonism needs a

peripheral safety package. And the negative-feedback problem of Section VIII applies with full force to any sustained agonist.

Kill criterion. If an engineered bivalent binder cannot produce sustained Disabled-1 phosphorylation and a downstream reduction in phospho-tau in a human-tau mouse, at exposures achievable with a shuttled antibody, the route is dead. That experiment is affordable and it is the first one to run.

Route Two — Splice-Switching at the ApoER2 Cytoplasmic Exon

Mechanistic proximity: moderate. Clinical de-risking: highest of the five.

ApoER2's short cytoplasmic tail carries an NPxY motif that docks Disabled-1,[35] and an alternatively spliced proline-rich cassette — exon 19 — whose inclusion is controlled by neuronal activity and which governs how strongly reelin signalling through the receptor potentiates the synapse and supports memory. Mice lacking the exon are impaired in learning and memory.[36]

The therapeutic observation is that this splicing balance is *deregulated in post-mortem human Alzheimer's brain* and in Alzheimer's model mice, and that a single dose of a splice-switching antisense oligonucleotide corrected it for six months while improving synaptic function, learning and memory.[37]

This is, by some distance, the most clinically de-risked entry on the list. Central-nervous-system antisense oligonucleotides are an approved modality with an established intrathecal delivery route; the blood-brain-barrier problem that dominates every other route does not arise. The target is human-validated in the sense that matters most — the abnormality being corrected is one demonstrably present in patients' brains. And a single dose held for six months.

Its weakness is honest to state: it is not a phenocopy of COLBOS. It tunes the receptor's *output* per clustering event rather than the *frequency* of clustering events. The variant works on the extracellular staging; this works on the intracellular gain. They converge on Disabled-1 phosphorylation from opposite sides of the membrane. Whether that convergence is therapeutically equivalent is untested, and could be tested by crossing the exon-19 manipulation onto the COLBOS knock-in.

Route Three — Inhibiting the Reelinase

Mechanistic proximity: moderate. Preclinical readout: already in hand.

Reelin is inactivated by proteolysis. Two canonical sites are mapped to the residue: an N-terminal cut between Pro1244 and Ala1245 within repeat 3, which "virtually abolishes" signalling activity,[38] and a C-terminal cut between Ala2688 and Asp2689 to which meprins contribute, though meprin-β is not the major basal enzyme.[39] A cleavage-resistant reelin remains active longer

and signals persistently.[40] A third cut, by a furin-family proprotein convertase six residues from the C-terminus, abolishes membrane binding and the neuropilin-1 interaction[9,10] — and, note, removes the α-site glycosaminoglycan determinant that sits immediately adjacent to the COLBOS residue.

The dominant N-terminal reelinase in cortex and hippocampus is ADAMTS-3, and the knockout experiment is close to a preclinical proof of concept for this route: N-terminal cleavage falls, Disabled-1 falls, dendritic branching increases, and **tau phosphorylation is significantly decreased**. The authors state the therapeutic implication themselves — that inhibiting ADAMTS-3 upregulates reelin activity and may be a therapeutic strategy.[14] In the adult cortex and hippocampus specifically, ADAMTS-2 contributes significantly,[41] which matters for a drug aimed at adult disease; ADAMTS-4 cleaves in an isoform-dependent manner,[42] and tissue plasminogen activator and ADAMTS-5 participate in a network modulated by tissue inhibitors of metalloproteinases and serpins.[43]

This route has a distinctive virtue: it acts on *endogenous* reelin, and it raises the pool of the signalling-competent, C-terminal-region-intact species — the species whose staging COLBOS improves. It is the only route on this list that increases the substrate the variant acts upon.

Liabilities. Metalloproteinase inhibition has a bruising clinical history of off-target musculoskeletal toxicity, and ADAMTS-family selectivity is demanding. And the overdrive caution applies directly here, since the same enzymatic deletion that reduces tau phosphorylation also produces developmental abnormalities from unopposed signalling.[14] A partial, titratable inhibitor is wanted, not a potent one.

Route Four — Rescuing the Receptor from the Endosome

Mechanistic proximity: moderate, and genotype-restricted.

In the ApoE4 carrier, ApoER2 is withdrawn from the surface into endosomes it recycles from poorly, and reelin-mediated synaptic modulation is correspondingly impaired. Inhibition of the sodium–hydrogen exchanger NHE6 restores it.[44]

The strategic significance of this result exceeds its mechanistic proximity. It is a *small molecule* that enhances reelin signalling while delivering no reelin — the most drug-like precedent in the entire space, and a demonstration that the pathway has small-molecule-addressable nodes even though its central protein–protein interface does not. Its restriction is that it corrects an ApoE4-specific lesion, so it is a genotype-targeted therapy rather than a general phenocopy. In a disease where ApoE4 carriage defines a large and high-risk population, that restriction is commercially and clinically tolerable.

Route Five — Tuning the Sugar Bed

Mechanistic proximity: highest of all. Tractability: lowest of all. Ranked last for that reason.

Formally, this is the most faithful phenocopy available. COLBOS improves reelin's engagement with N-sulfated heparan sulfate; the exactly corresponding intervention is to improve the sugar bed — raise N-sulfation, or supply N-sulfated mimetics that stage reelin more effectively. The 2025 study names the strategy in its closing sentence and hands the programme its determinant, its assay, and its structural rationale, including the docking-implicated arginines in the tail.[11]

The pharmacology does not cooperate. Nothing targeting heparan sulfate sulfation has entered a central-nervous-system trial. Every clinical-stage agent in the chemotype is oncological, intravenous, and selected for anti-heparanase or immunomodulatory activity rather than sulfation-pattern selectivity: pixatimod reached a maximum tolerated dose of a hundred milligrams in twenty-three patients with dose-limiting hypertension and epistaxis and no objective responses,[45] and roneparstat, an N-acetylated glycol-split heparin, reached phase I in myeloma.[46] These are large polyanions that do not cross the blood-brain barrier; the heparin mimetic used to block tau uptake in vivo worked only by stereotactic co-injection.[47] Anticoagulation and platelet toxicity dog the class. Chlorate, the global sulfation inhibitor, strips sulfation from every proteoglycan in the cell and is strictly a tool compound.

And the selectivity requirement is not merely difficult but, on present evidence, self-contradictory — which is the subject of the next section.

The Five Routes, Ranked

Rank	Route	Proximity to variant	Best precedent	Principal liability
1	Clustering agonist at ApoER2/VLDLR	Highest actionable	Bivalent anti-LBD agents fire Dab1 and raise LTP[28]	BBB delivery; Dab1 feedback; peripheral receptor biology
2	ApoER2 exon-19 splice-switching ASO	Moderate (gain, not frequency)	Single dose corrects splicing 6 months, improves memory[37]	Not a true phenocopy; intrathecal dosing
3	ADAMTS-3/ADAM TS-2 inhibition	Moderate (raises substrate)	KO lowers tau phosphorylation in vivo[14]	Metalloproteinase selectivity; overdrive pathology
4	NHE6 inhibition (ApoE4 arm)	Moderate, genotype-restricted	Small molecule restores reelin signalling[44]	Restricted to ApoE4 carriers
5	Heparan sulfate sulfation tuning	Exact	Determinant and assay defined[11]	No CNS-tractable chemistry; and see the knife-edge

VII. The Knife-Edge

We now name a collision between two literatures that, so far as we can determine, neither has acknowledged. It is the most consequential original observation in this dissertation, and it constrains not only Route Five but the entire field of heparan-sulfate-directed anti-tau therapeutics.

Pathological tau enters neurons through heparan sulfate proteoglycans. The foundational demonstration showed that heparan sulfate proteoglycans mediate tau fibril internalization and transcellular propagation by macropinocytosis, and that the process is blocked by heparin, by chlorate, by heparinase, and by knockdown of the polymerase EXT1 — with a heparin mimetic blocking neuronal uptake of injected tau fibrils in vivo.[47] The requirement was subsequently refined into a sulfation code: tau uptake requires defined N- and 6-O-sulfate moieties, and CRISPR knockout of NDST1 or HS6ST2 significantly reduces it.[48] A further layer concerns intracellular fate — the 3-O-sulfotransferase HS3ST2 is significantly upregulated in the Alzheimer's hippocampus, 3-O-sulfated heparan sulfate binds tau directly and drives abnormal phosphorylation, and knockdown rescues phospho-tau epitopes and motor phenotypes in zebrafish,[49] while HS3ST2 gain-of-function induces cell-autonomous tau aggregation.[50]

Set that beside the reelin result, term by term.

Perturbation	Effect on tau	Effect on reelin
Heparin	Blocks fibril uptake[47]	Blocks reelin-induced ApoER2 dimerization[11]
Heparinase	Blocks fibril uptake[47]	Reduces surface binding and dimerization[11]
NDST1 knockout	Reduces tau uptake[48]	Reduces reelin surface binding to ~30–40%[11]

Three of the field's principal anti-tau tools against the heparan sulfate route each independently silence reelin signalling. The overlap is not approximate or analogical; it is the same enzyme and the same reagents. The sugar that admits tau into the neuron is the sugar that stages the brake on tau.

And the trade-off is worse than symmetric, because of the variant. RELN-COLBOS binds heparan sulfate *more tightly* than wild-type reelin.[11] The resilience allele this dissertation proposes to imitate is *more* heparan-sulfate-dependent than the norm. A heparan-sulfate-blocking anti-tau drug would therefore not merely risk collateral damage to a protective pathway; it would act directly and specifically against the COLBOS mechanism. The better a patient's reelin staging, the more such a

drug would take from them.

We checked whether either literature has noticed. In the full text of the reelin co-receptor paper, tau appears once, in a reference, and never in the main text; the paper closes by proposing therapeutic strategies targeting heparan sulfate sulfation with no discussion of risk.[11] The tau-uptake literature, correspondingly, frames heparan sulfate targeting purely as benefit and does not mention reelin or any other heparan-sulfate-dependent signalling. Neither cites the other. We name the collision here, and we recommend that any heparan-sulfate-directed anti-tau programme measure reelin signalling as a safety pharmacology endpoint.

The knife-edge also cuts a third way, which sharpens rather than complicates it. Apolipoprotein E binds heparan sulfate through a groove lined by arginine-136 — the Christchurch residue — together with the basic cluster at 142 to 147, and the high-affinity complex requires N- and 6-O-sulfo groups of glucosamine.[51] All apolipoprotein E isoforms, including Christchurch, recognize 3-O-sulfated heparan sulfate, and knockout of the 3-O-sulfotransferase HS3ST1 reduces cell-surface apolipoprotein E binding and uptake.[52] Reelin, tau, and apolipoprotein E all read marks on the same polymer. This is the strongest available statement of the shared-node reading that the companion volumes of this corpus develop, and it is also a warning: the node is shared by the protective and the pathological alike, so nothing that acts on the node itself can be selective.

The design rule that resolves the knife-edge is therefore: target the protein that reads the sugar, never the sugar itself. An agent directed at a protein can distinguish reelin from tau from apolipoprotein E. An agent directed at the polymer cannot. This rule is not a speculation — it is the architecture of the one variant-mimetic agent that has already been validated in vivo, and Section IX takes it up.

One further note belongs here, because it connects this dissertation to the wider corpus. Reelin is secreted into perineuronal nets by cortical bitufted, horizontal and Martinotti cells, where it acts extrasynaptically.[53] Neurons bearing aggrecan-based nets are protected from tau pathology in human Alzheimer's brain, and the regions preferentially struck by tau — the nucleus basalis of Meynert, dorsal thalamus, raphe, and the locus coeruleus — are devoid of that matrix.[54] Perineuronal nets restrict both the distribution and the internalization of aggregated tau, demonstrated causally across aggrecan-, HAPLN1- and tenascin-R-null slices.[55] The matrix that stages reelin and the matrix that excludes tau are the same matrix, and the first nucleus to tangle is the one that lacks it.

VIII. Three Ways to Get It Wrong

A design brief is incomplete without its failure modes. Three are specific enough to state, and each has already produced a clinical or preclinical result the field should not have to learn twice.

The Distal Node

Reelin's brake on tau runs through Disabled-1 to phosphoinositide-3-kinase and Akt, and thence to inhibitory phosphorylation of glycogen synthase kinase-3β — genetically dependent on ApoER2, VLDLR and Disabled-1, and enriched in axonal growth cones.[56] The corresponding tau result is that the reelin/apolipoprotein-E-receptor/Disabled-1 complex suppresses tau phosphorylation, that glycogen synthase kinase-3β activity rises in reelin-null mice and rises further in reelin/apolipoprotein-E double-nulls, and that CDK5 activity and phosphatase activity are unchanged.[57] The brake is specifically a *kinase* brake.

The obvious inference — inhibit the kinase directly, and skip the pathway above it — has been tested and has failed. Tideglusib, a specific glycogen synthase kinase-3 inhibitor, was taken into a properly powered phase II trial in mild-to-moderate Alzheimer's disease: three hundred and six patients, fifty-five sites, twenty-six weeks, three dose arms. There were no statistically significant differences between any active arm and placebo on any efficacy variable, alongside diarrhoea in fourteen to eighteen per cent and dose-dependent reversible transaminase elevation in nine to sixteen per cent against three and a half per cent on placebo. The authors' own summary: acceptably safe, no clinical benefit.[58]

Honesty requires the counter-evidence. Lithium — weak, promiscuous, and not a specific inhibitor — failed over ten weeks in mild Alzheimer's disease with no effect on lymphocyte glycogen synthase kinase-3 activity, cerebrospinal fluid tau species, or cognition.[59] But two longer trials in amnestic mild cognitive impairment were positive: twelve months at subtherapeutic concentrations significantly reduced cerebrospinal fluid phospho-tau with better cognitive performance,[60] and a two-year double-blind extension found placebo declining while lithium-treated patients remained stable.[61]

The two readings converge on the same lesson for this programme, by different roads. The potent, specific, direct inhibitor of the distal node failed outright and was hepatotoxic. The weak, gentle, early, sustained modulator showed benefit only over years and only in prodromal disease. What a receptor-level intervention offers is precisely the second profile delivered by physiology rather than by pharmacological accident: a modest, tonic, spatially organized restraint on the kinase, applied where the receptors are clustered rather than uniformly throughout the cell. That is

an argument for acting upstream, and it is the strongest one available.

The Fyn Double Edge

Reelin regulates NMDA receptor activity through Src-family kinases and Disabled-1, and Src-family inhibition abolishes the reelin-induced calcium enhancement.[62] A reelin-pathway agonist therefore *activates* Fyn, downstream of Disabled-1, by design.

The field has spent a decade trying to do the opposite. Amyloid-β oligomers bound to cellular prion protein at the postsynaptic density activate Fyn, phosphorylate the NR2B subunit and drive spine loss.[63] Fyn became an attractive target on the reasoning that it uniquely links amyloid and tau pathology, with the acknowledged liability of broad expression.[64] The Src-family inhibitor saracatinib was taken into a phase 2a trial of a hundred and fifty-nine patients over fifty-two weeks and missed its primary endpoint, with no difference in cerebral metabolic decline and none in cognitive or functional measures.[65]

A programme proposing to activate Fyn in a disease where the field has been trying to inhibit it owes an account of the discrepancy. The account available is that spatial context, not net kinase activity, is what matters: reelin-driven Fyn activation is receptor-clustered and locally organized at growth cones and the postsynaptic density, whereas the amyloid-oligomer/prion-protein route generates a diffuse pathological pool. This is a coherent reconciliation and it is consistent with the anatomy of both signals. It is also **unproven, and this dissertation grades it as inference rather than evidence.** It is a first-order derisking experiment for Route One: does a clustering agonist produce the spatially restricted Fyn signature of reelin, or the diffuse one of amyloid oligomers?

The Self-Limiting Agonist

The third failure mode is intrinsic to the pathway and is the most easily overlooked. Reelin signalling triggers the tyrosine phosphorylation of Disabled-1 — and phosphorylated Disabled-1 is thereby marked for destruction. Its degradation requires that phosphorylation and requires the E3 ubiquitin ligase component Cullin 5, working with SOCS proteins that bind phospho-Disabled-1 and target it; ablating Cullin 5 in migrating neurons causes active Disabled-1 to accumulate and produces a distinct over-migration defect.[66]

The pathway destroys its own transducer as a condition of transducing. Any chronic agonist therefore faces tachyphylaxis by adaptor depletion, and a maximally potent agonist would deplete fastest. This argues for intermittent or pulsatile dosing rather than continuous saturation; for titrating to a partial effect, which the design brief already recommended on independent grounds; and possibly for combination with SOCS or Cullin-5 modulation, though that is speculative and carries its own developmental risk given the over-migration phenotype.

It also supplies an elegant retrospective explanation of why the variant is the shape it is. A germline gain of roughly twofold in co-receptor affinity, present from conception and acting for sixty-seven years, is precisely the sort of gentle, chronic, sub-saturating adjustment that a self-limiting pathway can sustain indefinitely. The variant is not a strong agonist. It could not have been one and still worked.

IX. The Mirror Programme

The strongest argument that a variant-phenocopy programme is executable is that one is already under way, in the same kindred, on the same receptor system, from the same investigators — for the other protected individual.

The Christchurch case has since been extended from a single homozygote[1] to a cohort: twenty-seven heterozygotes among one thousand and seventy-seven *PSEN1*-E280A carriers, with median onset of cognitive impairment at fifty-two years against forty-seven in matched non-carriers, relatively preserved glucose metabolism, and less vascular amyloid at autopsy.[67] A five-year delay for heterozygotes against three decades for the homozygote — a dose-response that a therapeutic programme can aim at, and a useful calibration of how much benefit a partial pharmacological effect might yield.

And the phenocopy has been built. Antibodies were raised against the human apolipoprotein-E–heparan-sulfate-proteoglycan interaction; the lead, 7C11, preferentially binds ApoE4 and disrupts the heparin–ApoE4 interaction; its Fab structure was solved; and in vivo it reduced apolipoprotein-E-induced tau pathology in a P301S retina model and curbed phospho-tau at Ser396 in the brains of **systemically treated** ApoE4 knock-in mice.[68]

Read that construction against the knife-edge of Section VII and its elegance is apparent. The antibody targets the *protein* side of the protein–sugar interface. The heparan sulfate bed is never touched. Reelin's staging is left intact — indeed a Christchurch-mimetic antibody and a reelin-pathway agonist are, in principle, combinable, since one removes a competitor from the sugar-facing side while the other improves engagement on it. This is the design rule instantiated, and it is the template Route One should follow.

Two cautions keep the mirror honest. First, the Christchurch mechanism is not purely a heparan-sulfate story: ApoE3-Christchurch also binds tau and the Wnt antagonist Dkk1 directly, reducing tau aggregation by routes independent of proteoglycan binding,[69] and induced-pluripotent-stem-cell-derived Christchurch astrocytes show *enhanced* heparan-sulfate- and LRP1-mediated tau uptake together with superior lysosomal clearance[70] — the opposite direction from the simple "protects by refusing tau" reading. A rigorous programme must say so. Second, the gene-therapy arm of the apolipoprotein-E mirror — an AAVrh.10 vector delivering ApoE2 into the cerebrospinal fluid of ApoE4 homozygotes — has reported dose-dependent cerebrospinal ApoE2 expression and reductions in tau species across fifteen dosed patients, but that report exists in conference abstracts and company communications rather than peer-reviewed literature, and this dissertation grades it accordingly.

The transferable conclusion is nonetheless firm. Taking a resilience variant from an *n* of one to a systemically dosed, structurally characterized, in-vivo-active therapeutic candidate is not a hypothetical exercise. It has been done once already, on the neighbouring ligand, in five years.

X. The Validity Ledger

Every claim on which this dissertation's argument rests is graded below. **Tier I** denotes direct human evidence or definitive genetic epistasis; **Tier II**, robust animal or primary-neuron evidence; **Tier III**, in vitro or heterologous-system evidence; **Tier IV**, inference, in silico work, or unrefereed report. The symbol ● marks claims we consider load-bearing for the therapeutic argument; ○ marks supporting claims; † marks claims we advance as inference and do not assert as established.

Claim	Grade	Basis	Caveat
RELN-COLBOS carrier resisted ADAD ~23 y with high amyloid, limited entorhinal tau	**I** ●	Human case with autopsy[2]	n = 1; sister protected less; confounds recorded
COLBOS does not alter reelin binding to ApoER2 or VLDLR	**III** ●	Cell-free binding[2]	Cell-free; not tested on membranes
H3447 lies in the 32-residue C-terminal region, outside the R3–6 fragment	**III** ●	Sequence and structure[4,5]	Unambiguous; arithmetic
COLBOS raises heparan sulfate affinity ~1.5–2×	**III** ●	SPR, ITC, BLI, glycan array[11]	Authors call it a fine-tuning, not a large gain
COLBOS raises neuropilin-1 affinity ~10×	**III** ○	Peptide binding[2]	Single study; peptide not full protein
N-sulfated heparan sulfate is required for full reelin-induced ApoER2 dimerization	**III** ●	Split-luciferase, heparinase, NDST1 KO[11]	HEK293T/MLEC; no neurons, no in vivo
C-terminal region is required for membrane binding and efficient signalling, not secretion	**III** ●	Deletion/replacement biochemistry[8]	Corrects the earlier secretion inference
C-terminal region deletion impairs Dab1 phosphorylation in vivo	**II** ○	Knock-in mouse[9]	Developmental readout
Reelin potency depends on multimerization, not single-site affinity	**III** ●	10× gain on oligomerization;[6] C2101A binds but cannot signal[12]	Two independent lines, same conclusion
Reelin–ApoER2 LA1 interface is ~350 Å2 per face	**III** ○	Crystal structure[7]	Argues against small-molecule agonism at that site

Claim	Grade	Basis	Caveat
Bivalent anti-receptor agents fire Dab1 and raise LTP	**III ●**	Fc-RAP and anti-LBD polyclonals[28]	Polyclonal; never engineered as therapeutic
ApoER2 exon-19 splicing is deregulated in human AD brain and correctable by ASO	**I/II ●**	Human post-mortem + mouse ASO[37]	Single group; needs replication
ADAMTS-3 deletion lowers tau phosphorylation in vivo	**II ●**	Genetic KO[14]	Also causes developmental abnormality
Reelin protein/CSF rises in AD while signalling falls	**I/II ●**	+40% cortex;[71] Dab1 phosphorylation reduced[72]	Direction is regional, not uniform — see below
Entorhinal reelin-expressing pyramidal cells are depleted in AD	**II ○**	Mouse + human confirmation[73]	Human arm qualitative
Reelin haploinsufficiency accelerates plaque and tau pathology	**II ●**	Genetic cross[74]	The causal arm of the resistance claim
Adult reelin loss sensitizes to amyloid-β synaptic toxicity	**II ●**	Adult conditional KO[75]	Establishes reelin as endogenous buffer
Heparan sulfate mediates tau uptake and propagation	**II/III ●**	Heparin, heparinase, chlorate, EXT1; in vivo mimetic[47]	In vivo arm by co-injection
N-sulfation (NDST1) required for both tau uptake and reelin signalling	**III ●**	Two independent literatures[11,48]	**The knife-edge; neither cites the other**
Reelin-pathway agonism activates Fyn, which the field has tried to inhibit	**I †**	Reelin→SFK;[62] saracatinib failed[65]	Spatial-context reconciliation is **inference**
Chronic agonism is self-limiting via Cul5/SOCS Dab1 degradation	**III ●**	Cul5 ablation accumulates active Dab1[66]	Design constraint, not a disqualifier
Direct GSK-3β inhibition has failed clinically	**I ●**	Tideglusib phase II, n = 306, negative[58]	Lithium in aMCI is positive[60,61] — qualify
A Christchurch-mimetic antibody works systemically in vivo	**II ●**	7C11 in ApoE4 knock-in[68]	The template for Route One
Net-bearing neurons resist tau; the LC is net-poor	**I/II ○**	Human AD;[54] causal in KO slices[55]	Links to the corpus's coerulean volumes

Claim	Grade	Basis	Caveat
RELN CDS (10,383 bp) exceeds AAV capacity ~2.2×	**III ●**	RefSeq; oversized genomes truncate[22]	Arithmetic; forecloses Door Two
COLBOS is a base-editable substitution class	**IV †**	Codon 3447 = CAT; A>G middle position	PAM availability **not assessed**
Five COLBOS carriers among Colombian oldest-old	**IV ○**	Case series[3]	Low-profile venue; uncontrolled; colour only

XI. The Case Against

A dissertation that argued only its own thesis would be an advocacy document. The case against this programme is substantial and is set out here at its strongest.

The human evidence is one man. Everything above is an elaboration of a single case report, extended by a sister who was protected half as much and confounded three ways, and by an uncontrolled case series in a minor venue. Single-case genetics has misled before. Until a second well-characterized carrier is described, or a loss-of-function reelin variant with accelerated disease is found, the causal claim rests on the mechanistic corroboration rather than on the epidemiology.

The mouse effects were sex-limited and dosage-limited. Enhanced Disabled-1 phosphorylation in the knock-in cerebellum was detected in males only, and detecting Disabled-1 and glycogen-synthase-kinase-3β changes required *homozygosity* — where the protected man was heterozygous.[2] The tau reduction in the P301L cross was likewise in males. A phenotype that needs two alleles in a mouse to be visible, and only in one sex, is a phenotype whose effect size in a heterozygous human is being inferred generously.

Correlation with reelin abundance runs the wrong way, and the paradox is not fully resolved. Reelin protein rises about forty per cent in Alzheimer's cortex with a matching messenger-RNA rise and a parallel cerebrospinal-fluid increase, and cerebrospinal reelin correlates positively with tau.[71] More ligand accompanies more disease. The reconciliation this corpus adopts — that bulk reelin rises while functional signalling falls, because the protein aggregates into amyloid-like deposits with age[76] and because the reelin-expressing entorhinal pyramidal population is specifically lost[73] — is supported, and the functional arm is directly measured: reelin-dependent induction of Disabled-1 phosphorylation appears reduced in Alzheimer's disease, amyloid-β oligomers co-immunoprecipitate with reelin and blunt reelin-induced ApoER2 internalization.[72] But "more ligand, less signal" remains a composite explanation assembled from several studies rather than a single demonstration, and a programme betting on it should fund the direct experiment: quantitative phospho-Disabled-1 in human Alzheimer's cortex, which to our knowledge has never been reported.

Overdrive has its own pathology. Excess reelin signalling is not benign. Cleavage-resistant reelin and ADAMTS-3 deletion produce their own developmental abnormalities,[14] and the discovery paper itself notes that a stronger hypermorphic effect than COLBOS's "may not support proper development." The therapeutic window may be narrow enough that hitting it reliably in a heterogeneous human population is the real difficulty.

Developmental versus adult action is unresolved. Reelin's most famous role is in building the cortex. The protected man had his variant from conception. It is possible — not demonstrated, but possible — that part of his protection was structural reserve laid down in development rather than an ongoing adult brake on tau. If so, an adult-onset drug cannot reproduce it. The evidence against this worry is real: adult reelin deletion sensitizes mice to amyloid-β synaptic toxicity with memory deficits at very low amyloid burden,[75] and adult reelin supplementation improves plasticity and memory in wild-type animals.[19] The pathway is demonstrably active and drivable in the adult brain. But "active in the adult" is not the same as "sufficient in the adult to reproduce a lifetime effect," and this remains the single largest scientific risk to the programme.

The clinical trial is the hardest kind. The endpoint is delay of onset in people who are not yet impaired. The population is presymptomatic or prodromal. The duration is years. This is the most expensive and slowest trial design in neurology, and it is what the mechanism demands.

And there is no programme to join. We searched the clinical trials registry directly: there are zero interventional reelin-directed therapeutic trials. Every registry hit is an observational genetic or biomarker study, mostly in psychiatry. What exists instead is research infrastructure built by the discovery group — a distributable knock-in mouse, a deposited structure of the C-terminal region, an expanded case series. That is the profile of a laboratory building toward a programme, not of a programme under way.

XII. A Development Plan

If the argument of this dissertation is right, the work is specifiable. What follows is the sequence a programme would actually run, ordered so that the cheapest disqualifying experiments come first.

Stage 0 — Close the human gap. Quantify phospho-Disabled-1, total Disabled-1, and ApoER2 surface expression in human Alzheimer's cortex, stratified by Braak stage and by *APOE* genotype, with entorhinal cortex as the anatomical priority. This experiment has apparently never been done, and the entire therapeutic rationale depends on its result. If reelin-pathway signalling is *not* reduced in human Alzheimer's brain, the programme does not have a target.

Stage 1 — Establish the assay cascade. Three readouts, all available. The split-luciferase ApoER2 dimerization assay[11] measures the clustering event directly and is the pharmacodynamic assay that matches the variant's mechanism. Disabled-1 tyrosine phosphorylation in primary cortical neurons is the proximate signalling readout the discovery paper used.[2] Phospho-tau in a human-tau line is the distal readout. The COLBOS knock-in mouse, which is distributable, is the positive control — the benchmark any candidate must approach and should not greatly exceed.

Stage 2 — Run the two lead routes in parallel. Route One: generate engineered bivalent and multivalent binders against the ApoER2 ligand-binding domain, using the 2004 polyclonals as the proof that the epitope class works,[28] and screen through the Stage 1 cascade for valency and epitope dependence. Determine whether ApoER2 has an autoinhibitory preligand state,[33] since that determines whether agonism requires conversion or aggregation. Route Two: replicate the exon-19 splice-switching result independently and test whether its benefit is additive with clustering agonism or redundant with it.

Stage 3 — Test the spatial-context hypothesis. The Fyn reconciliation of Section VIII is inference, and the programme should not proceed to development without testing it. Determine whether clustering agonism produces the spatially restricted Src-family signature characteristic of reelin or the diffuse pathological signature characteristic of amyloid oligomers. A negative result here is a serious problem for the entire receptor-agonism approach and should be found early.

Stage 4 — Characterize the feedback. Measure Disabled-1 depletion under continuous versus intermittent agonism, and establish the dosing interval that sustains pathway tone without exhausting the adaptor.[66] Design the dosing schedule from this result rather than from convenience.

Stage 5 — Solve delivery. Only after Stages 1 through 4 does the blood-brain-barrier problem need solving, and by then the payload is antibody-scale rather than reelin-scale, which is

why it is soluble. Apply transferrin-receptor shuttle engineering at the affinities established to favour transcytosis over lysosomal routing.[15,16,18]

Kill criteria, stated in advance. The programme should stop if: phospho-Disabled-1 is not reduced in human Alzheimer's cortex (Stage 0); engineered bivalent binders cannot sustain Disabled-1 phosphorylation without exhausting the adaptor (Stages 1 and 4); clustering agonism produces a diffuse rather than a localized Fyn signature (Stage 3); or the effective dose in a human-tau model requires exposures exceeding what shuttle technology delivers (Stage 5).

And one recommendation to the wider field, which is independent of whether this programme is ever run. Any therapeutic effort directed at heparan sulfate to block tau propagation should measure reelin signalling as a safety endpoint. The knife-edge of Section VII is real, it is unremarked in both literatures, and it means that a successful anti-tau sugar-blocker may be silently disabling one of the few endogenous brakes on the pathology it is trying to stop — most severely in the patients whose reelin staging is best.

XIII. Verdict

Could a drug recreate the protective aspects of the RELN-COLBOS variant?

Not by copying it. The literal phenocopy is foreclosed four times over, and the four foreclosures are instructive rather than merely discouraging. The protein is too large for any delivery technology that exists or is in prospect. The gene is more than twice the capacity of the vector, and oversized genomes truncate rather than merely package poorly. The one fragment small enough to vector ends seven hundred and eighty-three residues before the mutation, so a COLBOS fragment is not a hard construct but an impossible one. And the edit, while of the right chemical class, has no route into a human neuron.

But copying it was always the wrong ambition, and the reason is the finding that organizes this dissertation. The variant does not improve reelin's grip on reelin's receptor — its own discovery paper reports, in a result the field has largely passed over, that binding to ApoER2 and VLDLR is unchanged. It improves how reliably reelin is *staged* at the membrane: a roughly twofold better grip on the N-sulfated sugar that clusters the receptor, a tenfold better grip on the co-receptor that partners it. In a pathway whose potency comes from simultaneity rather than strength — where a monomeric fragment is nearly inert, where forced oligomerization gains tenfold, where a mutant with perfect receptor affinity and broken dimer architecture cannot signal at all — a gain in staging is a gain in everything that matters. The variant is not a better key. It is a better grip on the doorframe while the key is turned.

And a drug is not obliged to reach that geometry by reelin's route. Reelin needs co-receptors because a soluble protein of modest single-site affinity cannot cluster a receptor unaided. An engineered bivalent molecule is under no such handicap: it clusters by construction. That is why the 2004 demonstration that antibodies against the ApoER2 and VLDLR ligand-binding domains fire Disabled-1 and enhance long-term potentiation is the most important underexploited result in this field. It shows the last step of the variant's mechanism can be reached directly, in one move, by an agent that contains no reelin, requires no sugar bed, and needs no neuropilin. **The drug that best phenocopies this variant is the drug that least resembles the protein it phenocopies.**

How viable? Honestly graded: the biology is Tier I in its human warrant and Tier III in its molecular detail, the lead modality has a twenty-year-old proof of concept and no engineering behind it, the delivery problem is real but antibody-scale and therefore tractable, and the trial is a prevention trial with everything that implies. There is no company, no investigational new drug application, and no clinical programme — only a laboratory assembling infrastructure. Set against

that, the mirror programme in the same kindred took the neighbouring resilience variant from a single case report to a structurally characterized, systemically active antibody with in-vivo tau effects in about five years, by following exactly the design rule this dissertation derives independently from the knife-edge: target the protein that reads the sugar, never the sugar itself.

So the answer is yes, with the emphasis on the second clause: **a drug cannot be this variant, and does not need to be.** The design target is not a molecule but a geometry — the clustered, co-receptor-staged engagement of a lipoprotein receptor on a patch of dendritic membrane, held a little more reliably than it otherwise would be, for a very long time, in a person who has not yet fallen ill. Two men in Antioquia showed that reaching that geometry is worth roughly three decades. One of them did it with a sugar-binding tail fourteen residues from the end of a four-hundred-kilodalton protein. A drug will have to do it some other way.

An understudy is not a double. It does not need to be the same actor. It needs to hit the same marks.

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Prepared under the Organic Network Synthesis methodology. All primary citations were verified against PubMed at the time of writing. Claims graded IV in the Validity Ledger, and the spatial-context reconciliation of Section VIII, are advanced as inference and are marked as such throughout. The gene-therapy programme referenced in Section IX and the vectored-fragment study referenced in Section IV, Door Three, exist as conference communications rather than peer-reviewed reports and are graded accordingly.