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CAUSAL TARGET VALIDATION MUST SEPARATE MOLECULAR ASSOCIATION, BIOLOGICAL MEDIATION, DISEASE MODIFICATION, AND THERAPEUTIC INTERVENTION

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ABSTRACT

Target validation is often described as a progressive accumulation of evidence, yet pharmaceutical research frequently treats qualitatively different claims as though they were interchangeable. Statistical association, molecular prediction, experimentally observed mediation, disease modification, and successful therapeutic intervention answer different scientific questions and are vulnerable to different sources of error. Collapsing these levels can cause an associated locus to be presented as a causal gene, a perturbed cellular phenotype to be interpreted as disease modification, or a genetically supported mechanism to be assumed pharmacologically actionable. This article develops a proposed causal target-validation model that preserves these distinctions while allowing evidence to be integrated across human genetics, functional perturbation, context-specific omics, disease models, and pharmacology. The model organizes target validation into separable evidentiary levels connected by conditional inference gates rather than by an automatic linear progression. It further introduces a causal-threat register covering confounding, pleiotropy, linkage, context mismatch, perturbation fidelity, direction of effect, and genetic-to-pharmacological analogy. Target advancement is consequently treated as a bounded, reversible decision based on the weakest unresolved causal link rather than on a single score or performance measure. The proposed model is conceptual and has not been empirically validated as a predictive or operational decision system. Its usefulness will depend on the quality, independence, biological relevance, and transportability of the evidence supplied to each component. By separating what evidence supports from what it cannot establish, the model may improve the design, interpretation, and documentation of target-validation programs while reducing premature claims of therapeutic causation.

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Introduction

Target validation occupies an unstable conceptual position between biological discovery and therapeutic development. In computational and AI-based pharmaceutical science, targets are increasingly prioritized through genome-wide association signals, expression models, network scores, multimodal evidence platforms, perturbation predictions, and other data-intensive methods. These approaches can make target discovery more systematic, but they also create a risk of evidentiary compression: heterogeneous observations are transformed into one rank, confidence score, or target–disease association value. A high-ranking target may therefore appear more causally established than the underlying evidence permits. The central difficulty is not simply that evidence is incomplete. It is that different evidence types support different claims, and those claims cannot be substituted for one another without additional assumptions.

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Human genetic evidence illustrates both the value and the limits of causal enrichment. Target–indication pairs supported by human genetics have shown a higher probability of clinical progression or approval in retrospective analyses, but the magnitude of this advantage depends on the quality of causal-gene assignment, indication matching, development phase, and analytical design [1, 2]. Genetic support should therefore be interpreted as evidence that may improve the prior plausibility of a therapeutic hypothesis, not as a guarantee that target modulation will be efficacious, safe, technically feasible, or clinically meaningful. A statistically credible genetic relationship may still point to the wrong gene, reflect a pathway that cannot be reproduced pharmacologically, operate only in an inaccessible tissue, or produce unacceptable consequences when altered after disease onset.

The same distinction applies to target assessment more broadly. Robust target evaluation requires coordinated consideration of biological rationale, experimental validity, translational relevance, safety, tractability, patient context, and the credibility of the decision process [3]. No single component can carry the entire causal argument. Strong disease association cannot compensate for an unresolved target identity; convincing cellular perturbation cannot compensate for an irrelevant model system; and successful target engagement cannot establish benefit when the proposed mechanism does not modify disease. Consequently, target validation should not be represented as a unitary status that becomes true once an evidence threshold or computational score is crossed.

This article proposes a causal target-validation model that separates four non-equivalent claims: molecular association, biological mediation, disease modification, and therapeutic intervention. It treats causal-gene assignment, context transportability, direction of effect, perturbation fidelity, pharmacological congruence, and uncertainty as explicit interfaces between these claims. The contribution is conceptual rather than empirical. It does not introduce a validated scoring system, predict clinical success, or prescribe universal advancement thresholds. Instead, it provides an evidence-preserving structure for asking what has been established, what remains assumed, which causal link is weakest, and what additional evidence would be required before a target–disease hypothesis could justifiably move forward.

Why Association is Routinely Mistaken for Therapeutic Causation

Molecular association is a reproducible statistical relationship between a genetic or molecular feature and a phenotype under a specified study design. Genome-wide association studies have identified extensive relationships between human variation and complex traits, creating valuable entry points for biological investigation and therapeutic hypothesis generation [4]. However, an association signal generally identifies a genomic region rather than a complete causal chain. It does not necessarily reveal the causal variant, the affected gene, the relevant cell type, the direction in which gene function should be modified, the biological process that mediates the phenotype, or whether that process can be altered safely by a therapeutic modality. Association is therefore evidence of patterned co-variation, not a compressed statement of molecular mechanism or intervention efficacy.

The tendency to overinterpret association is reinforced by the apparent objectivity of statistical significance, replication, and predictive performance. A well-powered and reproducible association may still be shaped by linkage disequilibrium, phenotype definition, population structure, ancestry composition, measurement error, selection, or incomplete representation of genetic architecture [5]. These limitations do not make association evidence uninformative. They define what the evidence can legitimately establish. Replication may confirm that a signal is stable across datasets, while calibration may show that a model estimates risk consistently. Neither operation determines which biological route carries the effect or whether changing that route will modify disease. Predictive utility and causal specificity must therefore be evaluated separately.

A second source of confusion arises when post-association annotation is treated as mechanistic confirmation. Assigning an associated locus to the nearest gene, a gene expressed in the affected tissue, or a pathway enriched among prioritized genes can generate biologically plausible hypotheses, but plausibility does not establish mediation. Moving from association to function requires progressively resolving the candidate variant, affected gene, cellular context, molecular consequence, and downstream phenotype through functional and experimental evidence [6]. Each transition can fail independently. The associated variant may regulate multiple genes, a nominated gene may be expressed without carrying the causal effect, or a perturbation may produce a detectable molecular response that is unrelated to the disease pathway.

Integrative association methods can narrow hypotheses while preserving the same inferential boundary. Transcriptome-wide association studies, for example, can identify genes whose genetically predicted expression is associated with a phenotype, but shared regulatory variants, co-regulation, linkage, and inappropriate tissue selection can produce non-causal gene prioritization [7]. The phrase “expression-associated gene” should therefore not be silently converted into “disease-mediating target.” The proposed model treats this conversion as an explicit gate requiring causal-gene resolution, context compatibility, and direct perturbational support. **Table 1** organizes the evidence, constructs, and interpretation boundaries needed to develop why association is routinely mistaken for therapeutic causation within the article’s central argument.

Table 1. Evidence domains, core questions, scientific requirements, and interpretation boundaries for Why association is routinely mistaken for therapeutic causation

Construct or evidence domain	Core question	Scientific basis	Role in the article	Failure or overclaiming risk	Boundary statement

Statistical molecular association	Does a molecular or genetic feature reproducibly co-vary with a phenotype?	Appropriately designed association analysis, replication, phenotype quality, ancestry fit, and bias assessment	Establishes an initial target–disease signal that may justify further investigation	Statistical significance may be presented as evidence of mechanism or intervention benefit	Association establishes patterned co-variation under specified conditions; it does not establish a causal gene, mediator, or therapy
Variant-level resolution	Which variant or set of variants is most compatible with the observed association?	Fine-mapping, trans-ancestry evidence, functional annotation, and linkage analysis	Narrows the genomic source of the signal and informs gene-assignment hypotheses	A credible set may be described as if it contains a definitively causal variant	Variant prioritization remains probabilistic until supported by independent functional evidence
Candidate causal-gene assignment	Which gene carries the disease-relevant effect of the associated signal?	Coding evidence, colocalization, regulatory mapping, chromatin links, allele-specific effects, and perturbation	Connects a genomic association to a testable biological entity	Nearest-gene, expression-based, or score-based nomination may be treated as causal identification	Gene assignment is a hypothesis requiring convergent evidence and cannot be inferred from proximity alone
Molecular correlation or predicted expression	Is a molecular trait correlated with disease risk or predicted from genetic variation?	QTL analyses, transcriptome-wide association, proteomic association, and molecular prediction models	Generates hypotheses about candidate mediators and relevant molecular states	Shared regulation or correlated instruments may be mistaken for mediation	Molecular correlation can prioritize mediators but cannot establish that the molecular trait carries the disease effect
Biological mediation	Does changing the candidate target alter the predicted disease-relevant process through a specified pathway?	Direct perturbation, temporal ordering, rescue, pathway readouts, and orthogonal assays	Tests the proposed mechanistic link between target and disease biology	Any detectable perturbation phenotype may be interpreted as disease-relevant mediation	Mediation requires a defined causal chain and a relevant context; a cellular response alone is insufficient
Context compatibility	Does the evidence arise in the relevant ancestry, tissue, cell type, state, disease stage, and environment?	Context-specific omics, patient-derived systems, stratified analyses, and external replication	Qualifies whether association and functional evidence can be transported to the intended disease setting	Evidence from convenient tissues or resting cell states may be generalized to disease biology	Causal interpretation is conditional on biological and population context
Therapeutic analogy	Can the genetically or experimentally inferred effect be reproduced by a feasible intervention with the required direction, magnitude, timing, and tissue exposure?	Gain- and loss-of-function evidence, target engagement, dose–response, chemical–genetic concordance, exposure, and safety assessment	Separates causal biology from modality-specific therapeutic feasibility	Lifelong genetic effects or complete gene disruption may be treated as equivalent to acute pharmacological modulation	A causal biological target may still be non-tractable, unsafe, inaccessible, or pharmacologically non-equivalent

Levels of Evidence from Molecular Correlation to Intervention

A causal target-validation argument can be organized as a set of non-equivalent evidentiary levels rather than as one cumulative score. The first level concerns reproducible molecular association; the second concerns resolution of the causal variant and gene; the third concerns biological mediation; the fourth concerns disease modification; and the fifth concerns whether a specific therapeutic intervention can reproduce the desired effect. Genetics-led drug-repurposing research demonstrates why these levels must remain separate. Genetic associations can generate or strengthen therapeutic hypotheses, but useful translation depends on target mapping, direction of effect, indication alignment, and confirmation through evidence that differs in design and failure mode [8]. A target may therefore have strong support at one level and remain uncertain at the next.

Fine-mapping and colocalization occupy an intermediate position between association discovery and mechanistic validation. Fine-mapping estimates which variants are most compatible with an association after accounting for linkage structure, while colocalization evaluates whether molecular and disease traits may share a causal signal. Both can improve resolution, especially when multiple variants and molecular traits compete for interpretation, but neither method directly demonstrates that a nominated gene mediates disease or that manipulating it will produce therapeutic benefit [9, 10]. In the proposed model, these methods strengthen the causal-gene assignment layer. They do not permit the assignment layer to be skipped. Their outputs should remain probabilistic and accompanied by uncertainty about linkage, allelic heterogeneity, molecular-trait measurement, and tissue relevance.

Disease modification requires a stronger claim than molecular mediation. A target may regulate a disease-associated transcript, pathway, or cellular phenotype without changing disease initiation, progression, severity, or a clinically relevant disease process. Drug-target Mendelian randomization may help estimate whether genetically proxied modulation of a target influences disease, particularly when instruments are proximal to the target and when linkage, pleiotropy, instrument strength, and directionality are examined [11]. Even then, the estimate represents the consequences of inherited differences operating across much of the life course. It does not automatically reproduce the magnitude, timing, tissue distribution, reversibility, or molecular selectivity of a pharmacological intervention.

Therapeutic intervention is therefore not the highest numerical value on the same evidentiary scale; it is a distinct modality-specific claim. It requires evidence that a defined intervention engages the intended target, in the required direction, within the relevant tissue and time window, at an exposure compatible with benefit and acceptable harm. Failure at this level does not

necessarily invalidate all preceding levels. A drug may fail because it does not reach the tissue, engages additional targets, produces the wrong degree of modulation, or is administered after an irreversible disease process has occurred. Conversely, successful target engagement cannot rescue a weak causal chain. The proposed model consequently records the status and uncertainty of every level separately and treats movement between levels as conditional, reversible, and dependent on evidence designed to test the specific missing inference.

Proposed Causal Target-Validation Model

The proposed causal target-validation model organizes evidence into four principal states: molecular association, biological mediation, disease modification, and therapeutic intervention. These states are connected by conditional inference gates rather than treated as interchangeable entries in a cumulative score. The model begins with a precisely defined target–disease hypothesis and requires each evidentiary state to record its data source, causal assumptions, biological context, uncertainty, and prohibited interpretation. This structure follows the broader causal-inference principle that a causal claim is defensible only when its identifying assumptions, sensitivity to violations, and relationship to alternative explanations are made explicit [12]. The model is therefore an evidence-accounting architecture, not a validated predictor of target success.

The first gate separates association from biological mediation. Passing this gate requires more than replication or statistical prioritization; it requires credible variant-to-gene resolution, correspondence between the measured molecular trait and the proposed target function, and evidence that the relevant effect occurs in an appropriate cell, tissue, or disease state. The second gate separates biological mediation from disease modification and asks whether target alteration changes a disease-relevant process rather than only an intermediate molecular readout. Causal estimates must be interpreted through explicit assumption checks, falsification strategies, and comparison with convergent or conflicting evidence rather than accepted because they originate from a method labelled causal [13].

The biological-mediation state is strengthened by direct, directionally interpretable perturbation evidence. Single-cell genetic perturbation can expose target-dependent programs and interaction structure that observational association cannot resolve [14, 15]. Within the proposed model, such experiments are most informative when they test the predicted mediator, record temporal ordering, include appropriate negative controls, and incorporate rescue or orthogonal confirmation. A perturbation phenotype is not considered sufficient merely because it is statistically distinguishable. Its relevance depends on whether the perturbation faithfully represents target modulation, whether the readout lies on the proposed disease pathway, and whether the experimental context approximates the biological context in which the therapeutic claim is intended to hold.

The final gate separates disease modification from therapeutic intervention. It requires an explicit analogy between the causal biological effect and the intended modality, including direction, magnitude, duration, tissue exposure, target engagement, and foreseeable safety consequences. The model does not require all evidence streams to agree before any research can proceed; instead, it makes disagreement decision-relevant. Concordant evidence may support advancement, while discordance may indicate a conditional hold, a targeted evidence request, or de-prioritization. The output is therefore a bounded decision state accompanied by unresolved threats and evidence requirements rather than a declaration that the target is valid in an absolute sense. **Table 2** organizes the evidence, constructs, and interpretation boundaries needed to develop proposed causal target-validation model within the article's central argument.

Table 2. Components, relationships, uncertainties, and validation needs within Proposed causal target-validation model

Component or layer	Inputs	Core function	Expected output	Uncertainty or limitation	Validation requirement
Target–disease hypothesis definition	Target identity, disease or phenotype, proposed direction of modulation, patient or biological context	Specifies exactly what causal and therapeutic claim is being evaluated	Versioned and falsifiable target–disease hypothesis	Ambiguous target identity, indication breadth, or direction can contaminate every later inference	Independent review of target, phenotype, intervention direction, and intended decision context
Molecular-association state	GWAS, QTL, molecular association, omics correlation, computational prioritization	Establishes whether a reproducible target-related signal exists	Bounded association claim with provenance and uncertainty	Linkage, population structure, phenotype error, shared regulation, and model dependence	Independent replication and explicit association-quality assessment
Causal-gene resolution gate	Fine-mapping, colocalization, coding evidence, chromatin links, regulatory mapping	Tests whether the nominated target plausibly carries the associated effect	Candidate causal-gene assignment with competing explanations	Multiple variants or genes, correlated molecular traits, tissue mismatch	Orthogonal gene-assignment evidence and locus-specific uncertainty analysis
Biological-mediation state	Direct perturbation, pathway readouts, temporal evidence, rescue, genetic interactions	Tests whether target alteration changes a predicted disease-relevant biological process	Defined target–mediator–phenotype relationship	Assay artifacts, compensatory responses, incomplete perturbation, irrelevant models	Replication across independent assays and biologically relevant systems
Causal-threat register	Confounding assessment, pleiotropy diagnostics, negative controls, alternative instruments, model-system review	Records pathways by which the apparent effect could be non-causal or misassigned	Threat-specific uncertainty and targeted evidence requests	Some assumptions remain only partially testable	Sensitivity analyses, falsification tests, and explicit residual-risk statements
Disease-modification state	Human causal inference, disease models, longitudinal	Tests whether target alteration changes disease development,	Context-bounded disease-modification claim	Genetic–pharmacological mismatch, reverse causation, species	Convergence across human and experimental evidence

	phenotypes, clinically anchored processes	progression, or severity		differences, stage dependence	with disease-relevant outcomes
Therapeutic-intervention gate	Modality, target engagement, exposure, dose–response, pharmacodynamic readouts, safety evidence	Tests whether the desired biological effect can be reproduced by a feasible intervention	Modality-specific therapeutic hypothesis	Off-target effects, inadequate exposure, nonlinear response, timing and tissue constraints	Chemical–genetic concordance, target engagement, exposure–response, and safety assessment
Decision-state output	Component-level evidence, uncertainty, tractability, safety, reversibility, consequences of error	Translates separated evidence into a bounded portfolio action	Advance, conditional hold, targeted evidence request, or de-prioritize	Thresholds and error costs vary across portfolios and indications	Prospective evaluation of decision consistency, calibration, and reversibility

Figure 1 presents the causal target-validation ladder, showing how the article's key components and boundaries are connected within proposed causal target-validation model.

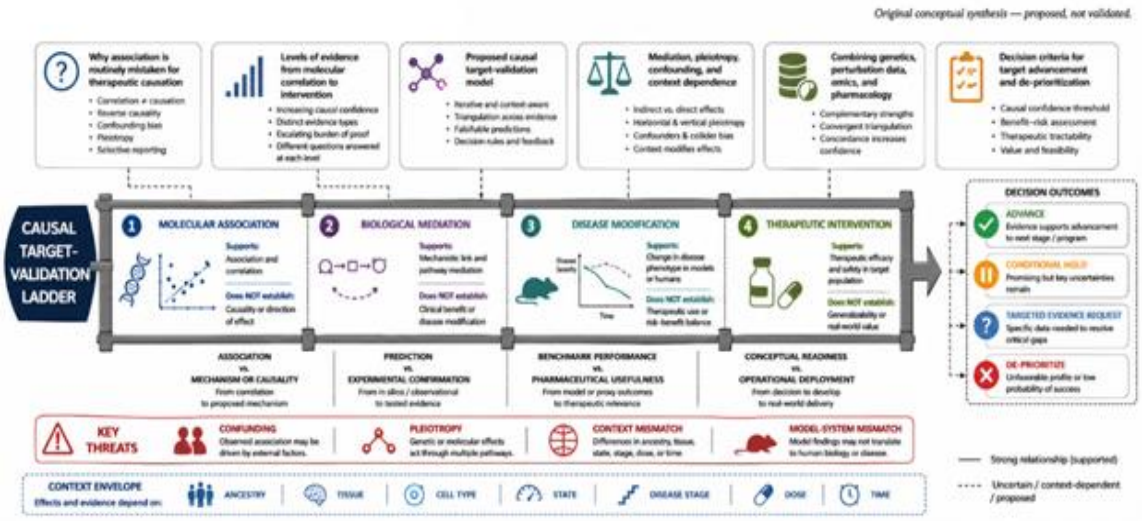

Figure 1. Causal Target-Validation Ladder.

The figure is an original conceptual synthesis that organizes the article's central contribution across association is routinely mistaken for therapeutic causation, levels of evidence from molecular correlation to intervention, proposed causal target-validation model, mediation, pleiotropy, confounding, and context dependence, context dependence, combining genetics, perturbation data, omics, and pharmacology. Arrows and grouping indicate proposed or evidence-supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

Mediation, Pleiotropy, Confounding, and Context Dependence

Mediation asks whether the effect attributed to a target operates through the biological process proposed by the target–disease hypothesis. In causal target validation, this question cannot be answered by observing that the target and mediator are correlated or that both relate to disease. The observed relationship may arise because the target influences another pathway, because the molecular traits share genetic determinants, or because a third process affects both. Mendelian-randomization estimates can likewise be distorted by correlated and uncorrelated pleiotropic effects, making it necessary to distinguish a target-specific causal route from broader genetic sharing [16]. The proposed model therefore treats mediation as a claim requiring a specified pathway and evidence capable of discriminating that pathway from credible alternatives.

Pleiotropy is especially consequential because complex-trait variants frequently influence multiple phenotypes, tissues, or molecular systems. Extensive shared genetic architecture means that a variant associated with both a molecular trait and disease does not necessarily imply that the molecular trait mediates the disease effect [17]. Vertical pleiotropy may be compatible with mediation when downstream consequences lie on the proposed causal pathway. Horizontal pleiotropy is more problematic because the instrument or variant influences disease through a pathway independent of the target or mediator being evaluated. The distinction is not merely terminological: it determines whether shared genetic evidence strengthens or undermines the intended target mechanism.

Pleiotropy and confounding should therefore be managed through a threat-specific diagnostic strategy rather than through one generic sensitivity label. Outlier detection, alternative instrument sets, multivariable analyses, negative controls, directionality tests, and comparison of methods with different assumptions may reveal instability compatible with horizontal pleiotropy. Empirical analyses have shown that such pleiotropy can be widespread in causal relationships inferred between complex traits and diseases, supporting formal diagnostic assessment before target claims are advanced [18]. Nevertheless, a negative

diagnostic cannot prove that all invalid pathways have been excluded. Residual uncertainty should remain visible and should guide the next experiment rather than disappear into a composite confidence score.

Context dependence qualifies every evidentiary level. A regulatory association observed in bulk tissue may be absent or reversed in a disease-relevant cell population, activated state, developmental period, or environmental condition. Disease-associated regulatory effects can be difficult to observe because available eQTL resources may not capture the cell types, exposures, or disease states in which those effects operate [19]. The model therefore requires a context statement covering ancestry, tissue, cell type, state, disease stage, dose, and time whenever relevant. Evidence should not be transported across contexts merely because the same gene name appears in each dataset. Context mismatch is treated as a causal threat that may warrant targeted evidence generation or restrict the scope of the claim.

Combining Genetics, Perturbation Data, Omics, and Pharmacology

Evidence integration is most informative when the contributing sources differ in their assumptions and failure modes. Human genetics may support direction and long-term disease relevance; perturbation experiments may test biological mediation; omics may resolve cell states and downstream pathways; and pharmacology may determine whether a feasible modality reproduces the intended effect. Computational models can predict single-cell perturbation responses and support transfer across measured conditions, but their outputs remain conditional on the representation of biological states in the training data and require experimental confirmation outside those conditions [20]. Prediction can prioritize experiments; it should not be relabelled as perturbational proof.

Chemical perturbation at single-cell resolution adds a different evidentiary dimension by revealing response heterogeneity, dose dependence, and state-specific effects that bulk averages can conceal [21]. Such data can identify whether a compound shifts the relevant cell population, whether the response is restricted to a substate, and whether increasing exposure changes the direction or breadth of the effect. These properties are directly relevant to therapeutic analogy. However, a transcriptional response in an in vitro system does not by itself establish disease modification, adequate tissue exposure, clinical efficacy, or acceptable safety. The model therefore records pharmacological-response evidence separately from disease-modification evidence.

Multimodal perturbation studies can strengthen mediation claims by measuring multiple consequences of target alteration in the same biological system. Combining pooled genetic perturbation with transcriptomic and protein readouts in patient-derived models can reveal mechanisms and state-dependent resistance phenotypes that would remain hidden in a single molecular layer [22]. Within the proposed model, multimodal agreement is valuable when the measured outputs lie on the hypothesized pathway and when their provenance remains explicit. Agreement among correlated readouts generated by the same assay system should not be mistaken for fully independent triangulation.

Matched chemical and genetic perturbations provide a further test of pharmacological congruence. Large-scale morphological profiling of cells exposed to paired chemical and genetic perturbations can reveal phenotypic similarity, divergence, and potential off-target activity [23]. Concordance may support a mechanism-of-action hypothesis when it is accompanied by target engagement, appropriate dose response, directional consistency, and orthogonal rescue. Discordance is equally informative because it can indicate polypharmacology, incomplete modulation, compensatory biology, or an incorrect target hypothesis. The proposed synthesis therefore uses evidence convergence as a structured comparison of provenance-preserving streams, not as a vote in which the largest number of datasets determines causality.

Decision Criteria for Target Advancement and De-Prioritization

Target advancement should begin with a precise definition of the efficacy-mediating molecular entity, the intended mechanism, the direction of modulation, and the indication. Mapping approved drugs to molecular targets has shown that target identity is often more complex than a simple gene label because therapeutic effects may depend on protein complexes, isoforms, binding sites, or context-specific mechanisms [24]. The proposed decision process therefore rejects advancement criteria framed only as “strong evidence for gene X.” The evaluated object must instead be a versioned target–mechanism–indication hypothesis with a stated modality and context.

Advancement should then be evaluated across separable dimensions rather than through a universal composite score. Biological rationale must be considered alongside tissue exposure, safety, patient selection, tractability, and the ability to test the proposed mechanism. Retrospective experience with multidimensional research-and-development frameworks supports the practical importance of considering these factors together, although such experience does not establish that one framework or threshold will be valid across organizations, therapeutic areas, or development stages [25]. In the proposed model, evidence dimensions may inform one decision while remaining individually visible; a strong value in one domain cannot silently compensate for failure in another.

Efficacy and safety should also be treated as distinct causal and translational questions. Analyses of clinical-trial stoppage indicate that weaker genetic support is associated with efficacy-related stopping, whereas safety-related stopping can additionally reflect properties such as target constraint, broad expression, and network connectivity [26]. A target with plausible disease-modifying biology may therefore remain unsuitable because intervention is likely to disrupt essential functions or produce effects outside the intended tissue. Conversely, a trial may stop because a modality fails to engage the target adequately even when the underlying target biology remains credible. De-prioritization should identify which evidentiary layer failed rather than collapsing every negative outcome into “invalid target.”

Integrated evidence platforms can support prioritization by preserving information about genetics, perturbation, pathways, known drugs, tractability, direction of effect, and safety. Such systems are valuable for hypothesis construction and comparison when the provenance and datatype of each evidentiary contribution remain accessible [27]. They should not be interpreted as engines that convert heterogeneous evidence into therapeutic causation automatically. The proposed model therefore permits four bounded outcomes: advance, conditional hold, targeted evidence request, and de-prioritize. Each outcome must state the decisive evidence, unresolved threats, reversibility conditions, and the consequence of being wrong. **Table 3** organizes the evidence, constructs, and interpretation boundaries needed to develop decision criteria for target advancement and de-prioritization within the article's central argument.

Table 3. Evaluation, implementation, and research priorities arising from Causal Target Validation Must Separate Molecular Association, Biological Mediation, Disease Modification, and Therapeutic Intervention

Evaluation dimension	What must be tested	Suitable evidence or assessment	Failure signal	Interpretive limitation	Decision relevance
Target identity	Whether the nominated molecular entity carries the disease-relevant effect	Fine-mapping, colocalization, coding evidence, regulatory mapping, direct perturbation	Competing genes or variants remain equally plausible	Probabilistic gene assignment does not prove mediation	Unresolved identity generally triggers a targeted evidence request rather than advancement
Biological mediation	Whether target alteration changes the proposed intermediate disease process	Directional perturbation, temporal ordering, pathway readouts, rescue, orthogonal assays	Perturbation affects unrelated pathways or fails to alter the predicted mediator	A cellular mediator may not modify disease in vivo	Strong mediation supports further validation but not therapeutic intervention by itself
Disease modification	Whether changing target function alters a disease-relevant process, progression, or severity	Human causal inference, disease models, longitudinal phenotypes, clinically anchored biomarkers	Effects are limited to correlational or non-disease readouts	Genetic effects may not reproduce acute pharmacology	Required before a target is treated as disease modifying
Direction and magnitude	Whether the therapeutic modality can reproduce the beneficial direction and sufficient degree of modulation	Gain- and loss-of-function studies, allelic series, dose–response, target engagement	Required modulation is nonlinear, excessive, or opposite to feasible pharmacology	Lifelong genetic exposure differs from time-limited intervention	Determines modality selection and may cause conditional hold
Context transportability	Whether the effect persists in relevant ancestry, tissue, cell type, state, disease stage, dose, and time	Context-specific omics, patient-derived systems, external replication, interaction analyses	Effect disappears, reverses, or becomes harmful in the intended context	Sparse context data may conceal heterogeneity	Restricts the scope of advancement and informs patient selection
Pleiotropy and confounding robustness	Whether the apparent causal effect could operate through an alternative pathway	Alternative instruments, multivariable analyses, negative controls, pleiotropy diagnostics	Estimates change materially across assumptions or instruments	No diagnostic excludes every invalid pathway	Persistent threats support targeted evidence generation or de-prioritization
Pharmacological congruence	Whether a modality reproduces target-specific genetic or biological phenotypes	Matched chemical–genetic profiling, exposure–response, target engagement, rescue	Chemical and genetic perturbations diverge without an explainable mechanism	Phenotypic similarity is not sufficient evidence of on-target action	Informs whether failure belongs to the target or modality
Safety and system dependency	Whether target modulation is compatible with acceptable consequences outside the intended pathway	Human loss-of-function evidence, constraint, expression breadth, network analysis, safety pharmacology	Essential-system disruption, broad expression, or adverse pathway activation	Absence of known risk is not evidence of safety	May override efficacy evidence and support de-prioritization
Decision calibration	Whether advance, hold, evidence-request, and stop decisions match uncertainty and failure costs	Prospective decision audit, blinded retrospective review, documented rationale and reversals	Opaque weighting, inconsistent thresholds, or persistent optimism bias	Portfolio-specific thresholds may not generalize	Evaluates the proposed model as a decision structure rather than a predictive score

Figure 2 presents the evidence, validation, and translation boundary observatory for causal target validation must separate molecular association, showing how the article's key components and boundaries are connected within decision criteria for target advancement and de-prioritization.

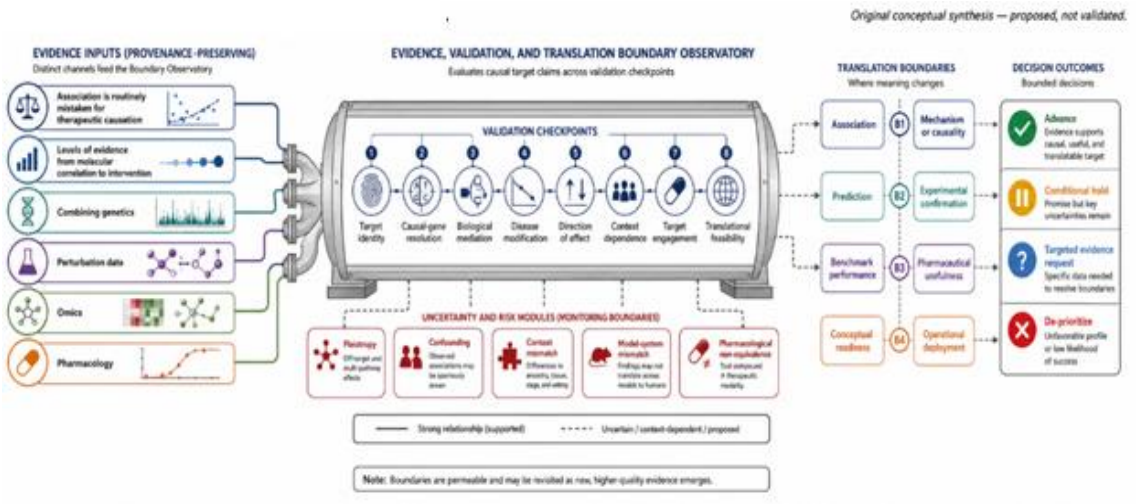

Figure 2. Evidence, Validation, and Translation Boundaries.

The figure is an original conceptual synthesis that shows how claims move from conceptual plausibility through evaluation, uncertainty assessment, and bounded pharmaceutical use. Arrows and grouping indicate proposed or evidence-supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

Limitations and Boundary Conditions

The proposed model is a conceptual contribution and has not been prospectively tested as a predictor of technical, clinical, or commercial success. Its components are grounded in established causal-inference and target-assessment principles, but the particular separation of evidence states, gate structure, threat register, and decision outputs requires empirical evaluation. Methods such as Mendelian randomization remain dependent on assumptions that cannot always be verified completely [12]. The model can make those assumptions visible and organize tests of their plausibility, but it cannot convert uncertain evidence into identified causality by documentation alone.

The framework is also limited by the evidence supplied to it. Relevant cell types, disease states, ancestries, developmental periods, and environmental conditions may be poorly represented, and a transparent model cannot compensate for missing biological context. Context-specific regulatory effects may remain unobserved in available molecular datasets [19]. Similarly, perturbation systems may not reproduce endogenous regulation, patient heterogeneity, long-term adaptation, or the exposure profile of a therapeutic modality. The resulting decision may therefore be carefully reasoned yet still wrong because the underlying evidence is unsuitable rather than merely incomplete.

A further boundary concerns implementation. The model should not be converted into an opaque weighted score that implies universal comparability among targets. Evidence dimensions differ in meaning, and advancement thresholds depend on indication severity, available alternatives, intervention reversibility, safety tolerance, and the consequences of false advancement or false rejection. Multidimensional decision frameworks can improve the visibility of such considerations, but experience from one portfolio cannot establish a universally valid threshold [25]. The model does not determine regulatory acceptability, clinical utility, reimbursement value, or deployment readiness, and it should not be used to bypass accountable scientific judgment.

Research Agenda and Implementation Priorities

The first research priority is to test whether independent evaluators can apply the proposed evidentiary distinctions consistently. Historical and active target programs could be annotated prospectively at the levels of association, candidate-gene assignment, mediation, disease modification, and therapeutic intervention. Evaluators should record unresolved assumptions, context restrictions, and the evidence that would change the decision. Perturbational evidence should be examined across multiple model systems because even information-rich single-cell screens remain conditional on assay design and biological context [14]. The purpose would not be to prove that one model predicts success universally, but to assess clarity, reproducibility, and the capacity to localize evidentiary failure.

A second priority is to create benchmarks that connect evidence across scales without treating one scale as a surrogate for another. These benchmarks should combine resolved genetic loci, direct perturbation, rescue, context-specific multi-omics, matched chemical–genetic profiling, target engagement, and disease-relevant outcomes. Chemical–genetic concordance should be evaluated against known on-target, off-target, and polypharmacological mechanisms so that similarity and divergence can be interpreted rather than merely scored [23]. Negative evidence, null perturbations, failed exposure, and adverse pathway activation should be preserved because they are essential for distinguishing target failure from modality or context failure.

Implementation should proceed in stages. The initial stage should focus on structured reporting: a versioned target–disease hypothesis, evidence-state summaries, a causal-threat register, context statements, and explicit advancement rationales. The next stage should test inter-rater consistency and retrospective decision calibration. Only after those studies should prospective decision-impact evaluation be considered. Integrated evidence infrastructures can support this process by retaining source, datatype, direction, and provenance [27]. Their role should be to make evidence inspectable and disagreements traceable, not to automate a declaration of therapeutic causation.

Conclusion

Causal target validation requires a disciplined separation of what is associated, what biologically mediates an effect, what modifies disease, and what can be reproduced by a feasible therapeutic intervention. The proposed model organizes these claims as distinct evidence states connected by conditional gates, a context envelope, and a causal-threat register. Its principal contribution is not a new score but a structure for preventing evidence from being interpreted beyond its legitimate boundary. A target may be genetically supported yet incorrectly mapped, experimentally active yet irrelevant to disease, disease modifying yet pharmacologically inaccessible, or pharmacologically engaged yet unsafe. By preserving these distinctions, the model may support more transparent advancement, targeted evidence generation, and de-prioritization. It remains a conceptual framework requiring prospective evaluation and should not be treated as a validated predictor, regulatory instrument, clinical recommendation, or deployment-ready decision system.

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