



Review Article

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## Rare Functional Variants Challenge Population-Based Rules for Precision Pharmacotherapy

David Osei<sup>1\*</sup>, Akua Afriyie<sup>1</sup>, Kofi Adu<sup>2</sup>

<sup>1</sup>Department of Pharmacogenomics and Rare Variants, Faculty of Pharmacy, University of Ghana, Accra, Ghana.

<sup>2</sup>Department of Precision Pharmacotherapy, Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

\*Email: [david.osei@ug.edu.gh](mailto:david.osei@ug.edu.gh)

### ABSTRACT

Pharmacogenomic implementation has largely been built around recurrent alleles whose population frequencies, functional effects, and clinical consequences are sufficiently characterized to support reproducible genotype-to-phenotype translation. Rare functional variants expose the limits of this architecture. Their clinical interpretation is difficult not simply because they are uncommon, but because variant detection, molecular function, pharmacological consequence, phenotype assignment, and prescribing actionability are separate inferential problems that often have unequal evidential support. Expanded sequencing can reveal variation omitted by targeted assays, yet additional detection frequently produces variants for which substrate-specific function, haplotype context, population distribution, or clinical consequence remains uncertain. High-throughput functional assays and computational annotation can narrow this gap, but assay readouts are not interchangeable and cannot automatically establish drug-specific clinical utility. Structural variation, reference-database imbalance, and ancestry-dependent discovery further complicate population-derived rules. This Current Opinion argues that rare-variant pharmacotherapy should therefore be organized around evidence transfer rather than population frequency alone. A proposed uncertainty-aware interpretation architecture distinguishes analytical detection, functional characterization, phenotype translation, and drug-decision evidence, while explicitly retaining discordance and unvalidated transitions. The central implication is that rarity should neither trigger automatic clinical action nor justify automatic dismissal. Precision pharmacotherapy instead requires variant-specific evidence interpreted within gene, substrate, drug, population, and decision-point boundaries.

**Keywords:** Pharmacogenomics, Rare variants, Functional genomics, Variant interpretation, Pharmacogenetic testing, Precision pharmacotherapy

### INTRODUCTION

Clinical pharmacogenomics is most mature where recurrent alleles can be identified reliably, translated reproducibly into phenotype categories, and connected to drug-specific prescribing recommendations. That model has been productive, but it creates an interpretive bias toward variants already frequent enough to have accumulated functional and clinical evidence. Population genomic analyses show that rare variation constitutes a substantial component of pharmacogene diversity, while its contribution differs markedly among genes and drug-response pathways [1, 2]. Rarity is therefore not a single biological category. It describes frequency, not functional magnitude, pharmacokinetic consequence, or clinical actionability.

The practical limitation becomes visible when sequencing is compared with targeted genotyping. In a large clinical pharmacogenomic comparison, sequencing identified potentially relevant variation that would not have

been represented by the modeled targeted approach, demonstrating that assay design can systematically restrict the observable variant space [3]. This does not mean that every additional sequence variant should alter treatment. It means that conventional pharmacogenomic rules are partly conditioned by what assays were designed to detect. Population structure introduces another layer. Clinically relevant pharmacogene alleles differ substantially in frequency across ethnogeographic groups, but population distributions cannot be substituted for an individual genotype or phenotype [4]. Broader sequencing can occasionally change metabolizer interpretation relative to commercial targeted testing, although such reinterpretation occurred only in a minority of individuals in the available comparison and does not itself demonstrate superior clinical outcomes [5].

The problem addressed here is consequently narrower than whether sequencing is “better” than genotyping. It is whether population-derived rules remain sufficient when a patient carries a rare variant whose analytical detection, molecular effect, phenotype translation, and drug consequence are supported to different degrees. The article argues that these levels should be separated explicitly. Population frequency can inform test design and evidence prioritization, but it should not determine functional importance by itself; functional evidence can strengthen interpretation without automatically establishing clinical actionability; and clinically meaningful decisions require evidence at the specific gene–drug–decision interface.

#### *Why rare variants escape conventional pharmacogenomic rules*

Rare variants escape conventional rules through more than low allele frequency. One source of failure is variant class. Pharmacogene variation includes copy-number changes, deletions, duplications, hybrid structures, and other configurations that cannot be reduced to a catalogue of single-nucleotide substitutions. Population analyses have demonstrated widespread copy-number variation across pharmacogenes, establishing structural variation as a distinct source of interindividual genomic diversity [6]. The consequence is methodological: a panel optimized for common single-nucleotide alleles can be complete within its intended design while remaining incomplete for the locus as a whole.

CYP2D6 represents the clearest example of this problem. Structural variation, duplicated alleles, hybrid configurations, and phasing requirements make phenotype assignment dependent on how the locus is interrogated and reported [7]. CYP2D6 should not be treated as representative of every pharmacogene, but it demonstrates why a frequency-based framework can fail before biological interpretation even begins. A rare sequence state may be misclassified because the underlying haplotype or copy-number structure is unresolved rather than because its functional effect is intrinsically unknowable.

A second failure occurs when analytical discovery exceeds interpretive capacity. DPYD illustrates this discovery–classification lag. Expanded genotyping or sequencing can identify rare variants beyond conventional test sets, but many do not possess sufficiently established functional evidence to support direct clinical interpretation [8]. The correct response is not to convert uncertain variants into presumed loss-of-function alleles merely because fluoropyrimidine toxicity is clinically important. Conversely, absence from a conventional panel should not be interpreted as evidence of functional neutrality.

Clinical laboratory recommendations already implicitly recognize that frequency is only one determinant of test design. TPMT and NUDT15 allele-selection recommendations incorporate functional evidence, population occurrence, technical feasibility, and availability of appropriate reference materials rather than using prevalence alone [9]. This is important for rare-variant pharmacotherapy because it separates two questions that are often conflated: whether an allele should routinely be included in a clinical test and whether that allele, once identified, has a sufficiently established biological or clinical consequence.

Functional follow-up can narrow uncertainty. Targeted next-generation sequencing combined with computational prioritization and in vitro characterization has demonstrated that previously uncharacterized CYP2D6 and CYP2C19 variants can be moved from detection toward experimentally supported functional interpretation [10]. Such workflows nevertheless stop short of proving clinical actionability. Enzyme activity measured in a model system is evidence about molecular function under specified conditions; it is not automatically evidence about therapeutic benefit, toxicity risk, dose requirement, or clinical outcome in every substrate context.

Rare variants therefore escape conventional pharmacogenomic rules through at least three distinct mechanisms: they may be absent from the assay, difficult to resolve structurally, or detected without sufficient evidence for translation. These are different failures and require different remedies. More sequencing addresses the first problem, improved locus resolution addresses the second, and orthogonal functional and clinical evidence addresses the third. Treating all three as a generic problem of “rare alleles” obscures where uncertainty actually enters the interpretation process.

### *Population frequency versus functional importance*

Population frequency remains useful because it influences which alleles are encountered, which variants merit inclusion in routine assays, and how well a reference database represents a given population. It becomes problematic when frequency is allowed to stand in for biological importance. Global CYP2C9 data illustrate that established functional alleles can show marked differences in distribution among populations [11]. These frequency differences matter for test coverage, but they do not redefine the molecular effect of an allele simply because it is common in one population and uncommon in another.

The converse problem is equally important: variants with similarly low frequencies need not have similar functional consequences. Functional characterization of rare SLCO1B1 variants demonstrated heterogeneous effects on OATP1B1-mediated transport, with variant-specific behavior that could then be explored through pharmacokinetic simulation [12]. The simulation component is useful for estimating possible exposure consequences, but it does not transform an in vitro finding into a validated prescribing recommendation. The central lesson is that rare-variant interpretation needs a functional axis independent of the frequency axis.

Population-based rules can also become incomplete when the canonical allele model does not capture all relevant locus-level information. In tamoxifen-treated patients, variation across the extended CYP2D6 locus contributed information about endoxifen concentrations beyond the conventional activity-score representation, although substantial variability remained unexplained and the finding should not be generalized to all CYP2D6 substrates [13]. This is not evidence that established phenotype systems are obsolete. It shows that a population-derived star-allele or activity-score framework can coexist with additional regulatory or locus-specific determinants.

For rare functional variants, frequency should therefore be interpreted primarily as an evidence-acquisition variable. High frequency increases the chance that function and outcomes will be characterized; low frequency reduces that opportunity. Neither condition determines the magnitude of the molecular effect. A very uncommon variant may have little measurable consequence, whereas another may strongly alter transport or metabolism. Precision pharmacotherapy requires those possibilities to remain analytically separate until functional and drug-specific evidence resolves them.

### *Functional assays and computational annotation*

The central technical opportunity in rare-variant pharmacogenomics is the ability to generate functional evidence at a scale that was previously impractical. Multiplexed variant-effect assays can interrogate thousands of pharmacogene variants, while studies of NUDT15 and CYP2C9 demonstrate how high-throughput measurements can distinguish variants with substantially different effects on protein abundance, enzymatic activity, or drug-related cellular phenotypes [14–16]. Their value is not that they create a universal functional score. Their value is that they replace absence of evidence with experimentally structured evidence for many variants that are too rare for conventional clinical association studies.

The meaning of a functional score depends on what is measured. For enzymes, abundance and catalytic activity may diverge. CYP2C9 mapping showed that reduced activity cannot be assumed to arise solely from reduced protein abundance [16]. For transporters, the same limitation becomes even more obvious: a high-throughput abundance measurement can identify variants that destabilize a protein, yet preserved abundance does not establish preserved transport. Deep mutational scanning of SLCO1B1 therefore supports scalable variant interrogation while simultaneously demonstrating the need for orthogonal, mechanism-matched validation [17]. Functional information also cannot automatically be transferred between homologous pharmacogenes. CYP2C19 deep mutational scanning revealed variant effects that were not simply predictable from corresponding CYP2C9 behavior, demonstrating that sequence similarity does not eliminate gene-specific structural and functional constraints [18]. This matters for rare alleles because the temptation to infer function by analogy increases precisely when direct evidence is sparse. Homology can inform prioritization, but it should not substitute for gene-specific characterization when the clinical inference depends on that distinction.

Computational annotation occupies a different evidential position. In silico tools are indispensable for prioritizing rare and noncoding variants because experimental characterization cannot immediately cover the full genomic search space. Their performance, however, depends on training data, variant class, annotation quality, and whether the predicted endpoint corresponds to the pharmacological function of interest [19]. A general deleteriousness prediction may indicate that a substitution is unusual or structurally disruptive without demonstrating altered metabolism of a specific drug.

CYP2D6 further shows why even direct experimental characterization can remain conditional. Rare CYP2D6 variants may display substrate-dependent effects across experimental systems, making a single universal activity

assignment biologically questionable for some alleles [20]. A variant that reduces metabolism of one substrate cannot automatically be assumed to produce an equivalent effect for another. Experimental system, substrate concentration, protein expression, and assay design become part of the evidential context rather than technical details that can be discarded after classification.

The proposed distinction here is between functional resolution and decision resolution. Functional resolution asks whether a variant alters an experimentally measured molecular property under specified conditions. Decision resolution asks whether that evidence is sufficiently transferable to a phenotype and a particular treatment decision. Multiplexed assays, orthogonal biochemical studies, and computational annotation can substantially improve functional resolution. They do not, by themselves, collapse the remaining distance to decision resolution.

#### *Variant classification under sparse clinical evidence*

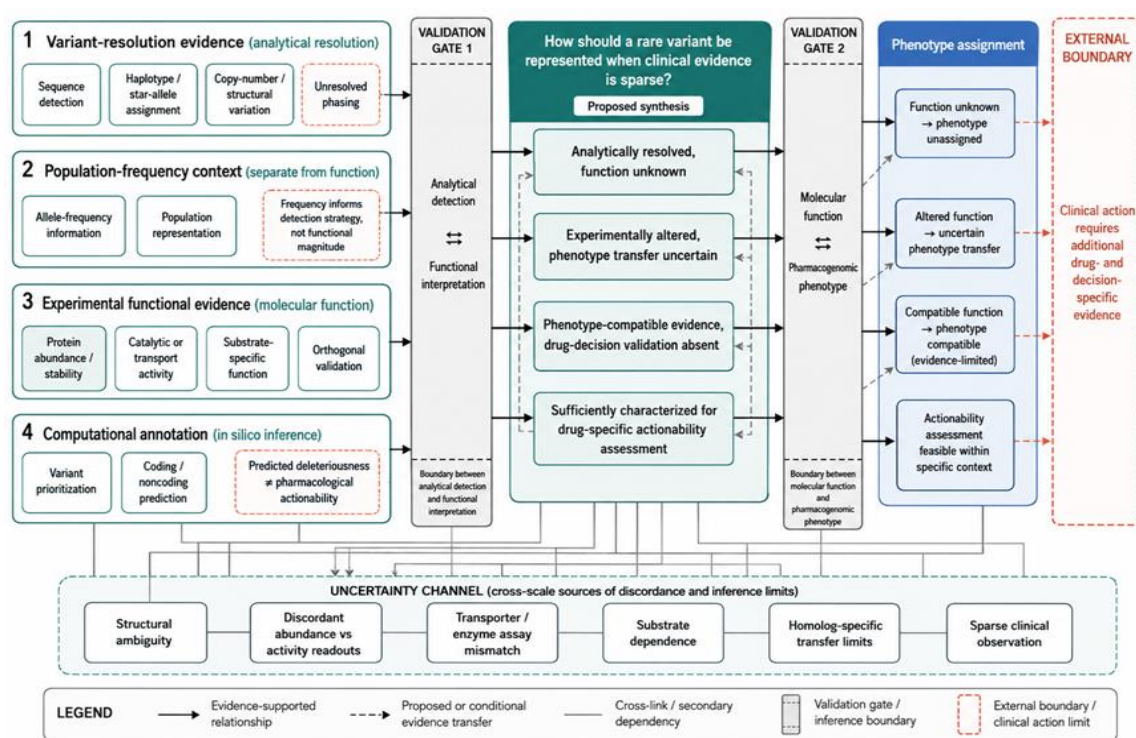
Variant classification becomes most difficult when molecular evidence is stronger than clinical evidence. Pharmacogenomics does not move directly from a sequence change to a prescribing rule. Even for a mature locus such as CYP2D6, genotype-to-phenotype translation requires a standardized interpretive system. International consensus work on CYP2D6 activity scores demonstrates that phenotype assignment is itself an inference layer designed to reduce inconsistent translation across laboratories and guidelines [21]. Standardization improves reproducibility, but it cannot manufacture evidence for an uncharacterized rare allele.

Mature gene–drug relationships provide an important comparator. TPMT and NUDT15 demonstrate that genotype-informed dosing can become sufficiently evidence based to support specific thiopurine recommendations when allele function, phenotype consequence, and treatment implications are established [22]. The existence of such examples should not be used to lower the threshold for uncharacterized rare variants. Rather, they show how much evidence separates detection from clinical actionability.

For rare variants, a binary actionable/non-actionable classification is therefore frequently too coarse. A variant may be analytically secure but functionally unresolved; functionally altered in vitro but not linked to a validated phenotype; compatible with reduced activity yet untested for the relevant substrate; or biologically plausible while still lacking sufficient clinical evidence for a treatment recommendation. “Uncertain” should likewise not be treated as a single state, because uncertainty can arise from assay limitations, structural ambiguity, discordant functional readouts, population underrepresentation, substrate specificity, or absence of clinical observations.

This article therefore proposes an evidence-transfer classification architecture in which four questions remain explicitly separate: Was the variant detected and resolved correctly? Is a molecular functional effect supported under relevant conditions? Can that effect be translated credibly into a pharmacogenomic phenotype? Is there sufficient drug- and decision-specific evidence for clinical action? The architecture is proposed rather than empirically validated as an integrated model. Its purpose is to prevent evidence accumulated at one level from being silently promoted to a stronger level.

The figure is needed here because the principal difficulty is not a shortage of evidence categories but the possibility of discordance among them: high-confidence detection may coexist with uncertain function, functional assays may disagree across readouts or substrates, and phenotype translation may remain provisional despite substantial molecular evidence.



**Figure 1.** Rare-variant evidence fractures and validation gates before pharmacogenomic phenotype assignment

#### *Ancestry and reference-database imbalance*

Rare-variant interpretation depends on what reference populations have been sequenced, functionally characterized, and incorporated into pharmacogenomic nomenclature and clinical testing systems. This creates an evidential problem that should not be confused with biological determinism. Reviews of pharmacogenomic evidence have documented persistent ancestry and sex representation imbalances, while broader multi-omics analyses show that historically excluded populations remain underrepresented in genomic resources used for precision medicine [23–25]. The relevant consequence is not that ancestry predicts an individual's pharmacological response. It is that unequal representation changes which variants are likely to have been discovered, named, experimentally studied, and connected to clinical evidence.

CYP2D6 diversity in sub-Saharan African populations provides a concrete example. Whole-genome analysis identified substantial haplotype and structural diversity, including predicted novel star alleles requiring further characterization [25]. Such findings expand the catalogue of variation that pharmacogenomic systems must accommodate, but most newly predicted alleles cannot immediately inherit functional or clinical meaning. Greater representation therefore increases both knowledge and the number of unresolved variants. Discovery and uncertainty can rise together.

Reference-database imbalance can distort interpretation in several ways. A variant may appear exceptionally rare because the populations in which it occurs are poorly represented. A haplotype may be difficult to assign because structural configurations are insufficiently catalogued. Computational predictors may have limited calibration for variant classes or genomic contexts poorly represented in their training resources. Clinical laboratories may lack reference materials for technically difficult alleles. Finally, clinical association evidence may remain sparse even when sequencing demonstrates that the variant is not exceptional in a particular population.

This article proposes the term uncertainty inequality for this uneven distribution of interpretive confidence across pharmacogenomic reference populations. The term does not imply that ancestry itself determines variant function. It describes a knowledge-state problem: equivalent analytical observations can carry different evidential support because discovery depth, nomenclature, functional characterization, and clinical follow-up have accumulated unevenly. The practical response is not to substitute ancestry for genotype, but to improve population representation while maintaining the same evidential boundaries for molecular and clinical inference.

More diverse sequencing alone is therefore insufficient. Newly observed pharmacogene variation must be connected to appropriate structural resolution, functional assays, reference materials, phenotype translation, and drug-specific evidence. Otherwise, expanded discovery can widen the gap between detectable variation and

interpretable variation. The design-space problem is summarized below because prose cannot show as efficiently how the source of discordance changes the appropriate monitoring requirement, alternative interpretive action, and unresolved question.

**Table 1.** How reference-population imbalance changes the interpretation space for rare pharmacogenomic variants

| Rare-variant inference scenario                                                                            | Evidence-certainty position                                                                  | Dominant source of discordance                            | Monitoring or verification requirement                                                                            | Appropriate alternative action                                                                               | Unresolved rare-variant question                                                                                  |
|------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Characterized functional allele observed in a population where its frequency is well represented           | Relatively high for allele identity and function; clinical certainty remains drug specific   | Residual uncertainty lies mainly beyond variant detection | Confirm genotype or haplotype quality and preserve drug-specific phenotype rules                                  | Use established allele-function translation while avoiding population-level assumptions about the individual | Does the established functional classification transfer to the exact drug and decision point under consideration? |
| Rare sequence variant detected in a well-sampled population but lacking direct functional characterization | Analytical certainty may be high while functional certainty remains low                      | Discovery exceeds molecular interpretation                | Seek orthogonal functional evidence matched to the pharmacogene and relevant molecular readout                    | Retain an unresolved or provisional functional state rather than assigning activity by rarity                | Which experimentally measured property is required before phenotype translation becomes credible?                 |
| Variant identified in a population poorly represented in pharmacogenomic reference resources               | Frequency estimates and prior evidence may be unstable even when the sequence call is secure | Reference-database underrepresentation                    | Verify population-specific occurrence, nomenclature, and available functional evidence                            | Separate uncertain frequency from uncertain function; do not infer either from ancestry alone                | Is the apparent rarity biological, database-driven, or both?                                                      |
| Complex haplotype, copy-number state, or structural configuration with incomplete catalogue support        | Function cannot be interpreted confidently until locus structure is resolved                 | Structural and phasing ambiguity                          | Apply locus-appropriate structural-resolution methods and confirm allele configuration                            | Delay phenotype assignment rather than collapsing the state into the nearest common allele                   | What functional unit has actually been inherited and expressed?                                                   |
| Newly discovered population-specific or population-enriched allele with predicted but unvalidated function | Computational support may exist while experimental and clinical certainty remain limited     | Uneven transition from discovery to validation            | Prioritize gene-appropriate functional testing and independent replication                                        | Treat computational annotation as supportive evidence, not as a prescribing classification                   | Does the predicted effect persist in the relevant assay, substrate, and haplotype context?                        |
| Concordant molecular evidence but little or no clinical observation in the relevant population             | Functional confidence may exceed decision confidence                                         | Sparse clinical exposure or outcome evidence              | Track drug, indication, concomitant therapy, organ-function context, and treatment phase where clinically studied | Preserve the functional interpretation while withholding unsupported drug-action claims                      | What additional evidence is necessary to move from plausible phenotype effect to a specific treatment decision?   |

Note: The scenario structure and distinction between discovery uncertainty, functional uncertainty, phenotype uncertainty, and decision uncertainty are proposed analytical syntheses of the evidence discussed in the manuscript. The table does not assign quantitative certainty grades or population-specific treatment rules.

### Translating rare variants into drug decisions

Clinical actionability should be assigned at the level of a drug–phenotype–decision combination rather than to a pharmacogene in the abstract. CYP2C19-guided clopidogrel therapy illustrates this specificity: metabolizer phenotype can support treatment modification in defined clinical contexts, but the recommendation belongs to clopidogrel use rather than to every CYP2C19 substrate [26]. Statin pharmacogenomics provides a complementary example because SLCO1B1, ABCG2, and CYP2C9 evidence differs by statin and by the exposure or safety question being considered [27]. The same functional allele can therefore carry different decision weight across drugs within one therapeutic class.

Psychiatric pharmacogenomics further shows that the presence of a gene–drug association does not guarantee a prescribing recommendation. Guidance for serotonin reuptake inhibitors differentiates relationships with sufficient evidence from pharmacodynamic associations for which evidence remains inadequate [28]. The 2024 beta-blocker guideline makes the asymmetry especially clear: CYP2D6 informs metoprolol recommendations, whereas evidence was insufficient for several other evaluated gene–beta-blocker relationships [29]. Insufficient evidence should not be rewritten as evidence of no effect; it marks a decision boundary.

Rare variants add another layer because the genetically inferred phenotype itself may be context sensitive. Large-scale psychiatric pharmacogenomic analysis showed that allele-specific copy-number refinement and phenoconversion can alter phenotype interpretation in subsets of patients [30]. Thus, even after a rare variant is functionally classified, drug choice, concomitant inhibitors or inducers, organ function, treatment phase, and the clinical endpoint remain relevant to actionability. The proposed decision rule is therefore conservative: escalate evidence only when each transfer—from variant to function, phenotype, and drug-specific decision—is justified.

#### *Uncertainty-aware clinical interpretation*

Uncertainty should be localized rather than collapsed into a single “variant of uncertain significance” label. A secure sequence call can coexist with uncertain haplotype structure; a reproducible molecular effect can coexist with uncertain phenotype translation; and a plausible phenotype can coexist with insufficient evidence for prescribing. CYP2D6 substrate dependence demonstrates why functional uncertainty may persist even after in vitro characterization [20]. Computational annotation should likewise remain supportive when direct pharmacological validation is absent [19].

This article proposes four distinct uncertainty domains: analytical resolution, functional interpretation, phenotype translation, and clinical decision evidence. These domains can be discordant rather than sequentially complete. Consensus phenotype systems reduce interpretive inconsistency but do not eliminate uncertainty for novel alleles [21]. Mature thiopurine guidance shows what becomes possible when several layers align [22], whereas beta-blocker guidance shows that actionability may remain drug specific even after a pharmacogene is well characterized [29].

#### *Research and implementation priorities*

Rare-variant pharmacogenomics should prioritize evidence generation according to the consequence of uncertainty, not frequency alone. Variants affecting drugs with narrow therapeutic margins, strong exposure–response relationships, or credible functional disruption may justify earlier orthogonal characterization than equally rare variants with little plausible decision consequence. This is a proposed prioritization principle, not a validated scoring system.

Implementation also requires a paired expansion of population representation and interpretive infrastructure. More diverse sequencing can reveal previously uncatalogued alleles, but discovery must be accompanied by structural resolution, reference materials, gene-appropriate functional assays, and transparent phenotype rules [25]. High-throughput functional methods can accelerate characterization [14], yet prospective clinical utility remains a separate evidential question. Programs should therefore report analytical validity, functional validity, phenotype inference, and decision utility separately rather than treating successful variant detection as implementation success.

## **CONCLUSION**

Rare functional variants expose a structural limitation of population-based pharmacogenomic rules: frequency, function, phenotype, and actionability are related but non-equivalent dimensions. Population data remain valuable for test design and evidence prioritization, while functional assays and computational tools can reduce uncertainty that clinical association studies cannot readily resolve for uncommon alleles. The proposed evidence-transfer architecture preserves those gains without allowing molecular evidence to outrun clinical inference. Rare variants

should neither be dismissed because they are uncommon nor promoted to treatment rules because they appear deleterious. Precision pharmacotherapy becomes more defensible when each variant is interpreted within its gene, assay, substrate, population, treatment, and decision context, with unresolved transitions made explicit rather than hidden inside a categorical phenotype label.

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