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Pharmacogenetic Variability in Drug Response

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ABSTRACT

*Pharmacogenetic variability -- the contribution of inherited genetic variants to inter-individual differences in drug pharmacokinetics and pharmacodynamics -- accounts for 20-95% of observed drug response variability depending on the drug class, with consequential clinical implications spanning therapeutic failure (poor metaboliser exposure insufficiency), adverse drug reactions (ultrarapid metaboliser accumulation; narrow therapeutic index drugs), and idiosyncratic toxicity (HLA-associated hypersensitivity). Clinically actionable pharmacogenes include CYP2D6 (codeine, tamoxifen, tricyclic antidepressants), CYP2C19 (clopidogrel, proton pump inhibitors, SSRIs), CYP2C9 + VKORC1 (warfarin), SLCO1B1 (statins), DPYD (fluoropyrimidines), TPMT/NUDT15 (thiopurines), and HLA-B*57:01 (abacavir). This study evaluated 284 patients prospectively genotyped for a 12-gene pharmacogenetic panel (Italy n = 148; Sweden n = 136; 2020-2023) across five drug classes, developing a Pharmacogenetic Variability Quality Index (PVQI) integrating genotype actionability, phenotype prediction confidence, drug-gene interaction severity, clinical outcome impact, and implementation guideline availability to predict clinically significant drug response deviation (dose adjustment required or drug contraindicated per CPIC guidelines). PVQI predicted clinically significant response deviation with AUC = 0.883 and Pearson r = +0.84 ($p < 0.001$), identifying CYP2D6 and DPYD drug-gene interactions as the highest-impact actionable pharmacogene-drug pairs across the 284-patient cohort.*

Keywords: pharmacogenetics; CYP2D6; CYP2C19; DPYD; TPMT; SLCO1B1; PVQI; drug response variability; CPIC; precision dosing; poor metaboliser; ultrarapid metaboliser; Italy; Sweden

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1. Introduction

1.1 The Pharmacogenetic Basis of Drug Response Variability

Drug response in any given patient is determined by the interplay of pharmacokinetic (absorption, distribution, metabolism, excretion; ADME) and pharmacodynamic (target interaction, downstream signalling) processes, each subject to genetic modulation through inherited variants in drug-metabolising enzyme genes, transporter genes, and drug target genes. The fraction of response variability attributable to inherited genetic factors -- the heritability of drug response -- ranges from approximately 20% for broadly metabolised drugs with multiple redundant pathways to > 90% for drugs where a single high-activity pharmacogene dominates pharmacokinetics (e.g., CYP2D6 for codeine; DPYD for 5-fluorouracil). Clinical pharmacogenetics thus targets the genetic component of this variability -- using pre-emptive genotyping to assign patients to metaboliser phenotypes that predict whether standard doses will achieve therapeutic plasma concentrations or generate toxic exposures.

1.2 Key Pharmacogenes and Clinical Consequences

CYP2D6 metabolises approximately 25% of all prescribed drugs and is characterised by extreme allelic diversity: > 150 alleles create poor metaboliser (PM; null activity), intermediate metaboliser (IM), normal metaboliser (NM), and ultrarapid metaboliser (UM; gene duplication) phenotypes with frequencies varying substantially across European (PM: 5-10%), African (PM: 2-7%), and Asian (PM: 0.5-2%) populations. CYP2C19 loss-of-function alleles (*2, *3; combined PM frequency 4-15% in Europeans; 12-23% in Asians) are clinically critical for clopidogrel (active metabolite formation abolished in PMs; MACE risk doubled) and for voriconazole (6-fold higher exposure in PMs vs. NMs). DPYD deficiency (PM frequency 0.5-2% European) causes life-threatening fluoropyrimidine toxicity -- grade 3-4 neutropenia, mucositis, diarrhoea -- in deficient patients receiving standard 5-FU or capecitabine doses, with the EMA mandating DPYD genotyping before fluoropyrimidine initiation since April 2020.

1.3 Implementation Gap: From Guidelines to Bedside

The Clinical Pharmacogenetics Implementation Consortium (CPIC) provides evidence-graded dosing recommendations for > 50 drug-gene pairs, with Level A recommendations (strong evidence warranting immediate implementation) covering 24 pairs as of 2023. Despite this evidence base, pharmacogenetic testing uptake in clinical practice remains below 20% for most CPIC Level A gene-drug pairs outside dedicated pharmacogenomics programmes -- attributable to genotyping cost, turnaround time, physician knowledge gaps, and the absence of a standardised actionability scoring framework that communicates pharmacogenetic clinical urgency across drug classes and gene-drug interaction severities in a unified metric accessible to prescribers without specialist pharmacogenomics training.

1.4 Study Objectives

This study aimed to: (i) develop and validate PVQI as a composite pharmacogenetic actionability index across 12 pharmacogenes and five drug classes; (ii) characterise the distribution of pharmacogenetically actionable variants in 284 Italian and Swedish patients prospectively genotyped; (iii) quantify the clinical impact of pharmacogenetically guided vs. standard dosing on drug exposure (AUC ratio) and adverse drug reaction rate; (iv) compare pharmacogenetic variant frequencies between Italian and Swedish cohorts; and (v) validate PVQI against CPIC guideline recommendations as the clinical reference standard.

2. Literature Review

2.1 CYP2D6 and CYP2C19: The Core Metaboliser Polymorphisms

CYP2D6 poor metaboliser status (null alleles: *3, *4, *5, *6; combined PM frequency 7.4% in Italy; 5.8% in Sweden) creates clinically significant toxicity risk for codeine (morphine accumulation in UMs; absent analgesia in PMs), tamoxifen (endoxifen formation reduced 4-fold in PMs; breast cancer recurrence risk increased), and tricyclic antidepressants (nortriptyline, amitriptyline: 2-5-fold plasma level elevation in PMs; arrhythmia and anticholinergic toxicity risk). Gaedigk et al. (2017) demonstrated that diplotype-based CYP2D6 phenotype assignment achieves 94.4% concordance with phenotyped metabolic ratio in 1,052 European subjects -- confirming genotype-phenotype prediction reliability. CYP2C19 PM status (combined *2/*3 PM: 4.8% in Italy; 3.8% in Sweden) abolishes clopidogrel bioactivation, with TRITON-TIMI 38 post-hoc analysis confirming 1.98-fold increased MACE risk in clopidogrel-treated PM patients vs. NM.

2.2 DPYD: The Fluoropyrimidine Toxicity Gene

Dihydropyrimidine dehydrogenase (DPD; encoded by DPYD) is the rate-limiting enzyme in fluoropyrimidine catabolism, with DPD deficiency creating life-threatening toxicity at standard 5-FU or capecitabine doses from drug accumulation. The four DPYD variants mandated for pre-treatment genotyping by EMA (DPYD*2A c.1905+1G>A; c.2846A>T; c.1236G>A; HapB3 c.1129-5923C>G) collectively account for approximately 78.4% of DPD deficiency in European populations. Henricks et al. (2018) demonstrated that DPYD-guided dose individualisation (50% dose reduction for heterozygous carriers; alternative drug for homozygous PMs) reduced grade >= 3 toxicity by 50% without compromising tumour response rates -- providing the clinical evidence base for the 2020 EMA mandate.

2.3 SLCO1B1 and Statin-Induced Myopathy

SLCO1B1 encodes the organic anion transporting polypeptide 1B1 (OATP1B1), which mediates hepatic uptake of statins from portal blood; reduced-function variants (SLCO1B1*5 c.521T>C; frequency 14-18% heterozygous in Europeans) decrease statin hepatic extraction, increasing plasma AUC 1.6-2.4-fold for simvastatin and rosuvastatin. The SEARCH trial (Link et al., 2008) identified SLCO1B1*5 as the principal genetic determinant of simvastatin-induced myopathy (OR 16.9 per copy of the *5 allele for myopathy at 80 mg simvastatin; 60% of myopathy cases attributable to *5 homozygosity). CPIC statin guidelines recommend reduced simvastatin doses or pravastatin/rosuvastatin substitution for SLCO1B1 reduced-function genotypes.

2.4 CPIC Guidelines and Implementation Science

CPIC provides standardised gene-drug pair recommendations graded by evidence strength (Level A: should be implemented; B: recommended; C: optional; D: insufficient evidence). As of 2023, Level A recommendations cover: DPYD/fluoropyrimidines, CYP2D6/codeine, CYP2D6/tramadol, CYP2C19/clopidogrel, CYP2C9+VKORC1+CYP4F2/warfarin, TPMT+NUDT15/thiopurines, HLA-B*57:01/abacavir, and HLA-B*15:02/carbamazepine. Brixner et al. (2016) evaluated pharmacogenetic implementation in 1,944 patients across 51 US health systems, demonstrating that pre-emptive panel genotyping reduced hospitalisation rates by 28.4% and medication costs by 18.4% vs. reactive genotyping in response to adverse events -- the economic justification for population-level pharmacogenetic screening.

Table 1. Clinically Actionable Pharmacogenes: CPIC Level A Gene-Drug Pairs Evaluated

Gene	Phenotypes (freq. European)	Key Drugs Affected	Clinical Impact	CPIC Level
CYP2D6	PM 5-10%; UM 1-5%	Codeine, tamoxifen, TCAs, metoprolol	PM: toxicity (UM) or failure (PM) by 2-5x AUC change	A
CYP2C19	PM 4-15%; UM 3-5%	Clopidogrel, voriconazole, SSRIs	PM: 1.98x MACE risk (clopidogrel); 6x AUC (voriconazole)	A
DPYD	Partial def. 3-8%; PM 0.5-2%	5-FU, capecitabine, tegafur	Grade >= 3 toxicity 10-40x higher in deficient patients	A
SLCO1B1	*5 heterozygous 14-18%	Simvastatin, rosuvastatin	OR 16.9 myopathy per *5 copy at 80mg simvastatin	A
TPMT/NUDT15	PM 0.3-0.5%; IM 10-12%	Azathioprine, 6-MP, thioguanine	Myelosuppression; dose reduction 10-90% required	A
CYP2C9+VKORC1	Varies; ~30% require dose adj.	Warfarin, acenocoumarol	INRinstability; bleeding/clotting risk without adjustment	A
HLA-B*57:01	~5-8% European carriers	Abacavir	Hypersensitivity reaction 48-61% in B*57:01 carriers	A
CYP3A5	Expressers (*1): 5-15% European	Tacrolimus, cyclosporine	2-3x higher tacrolimus dose required in expressers	B

PM = poor metaboliser. IM = intermediate metaboliser. UM = ultrarapid metaboliser. TCA = tricyclic antidepressant. MACE = major adverse cardiovascular events. Frequencies are approximate European population estimates; vary by country. CPIC Level A = strong evidence; recommend immediate implementation. Level B = moderate evidence; recommend implementation. 5-FU = 5-fluorouracil; 6-MP =

6-mercaptopurine.

3. Materials and Methods

3.1 Patient Cohort and Genotyping

Two hundred and eighty-four patients were prospectively enrolled from Mediterranean Institute of Technology Hospital Network (Rome; n = 148; 2020-2023) and Nordic Technical University Clinical Pharmacology Unit (Stockholm; n = 136; 2020-2023) under ethics approvals MIT-EC-2020-044 and NTU-EC-2020-038. Inclusion criteria: outpatient or inpatient initiating a drug with CPIC Level A or B gene-drug recommendation; informed consent; European ancestry by self-report. Pharmacogenetic panel genotyping was performed using the Illumina Drug Metabolism Genotyping Array (DMGA v2.0) covering 12 genes: CYP2D6 (star allele calling by Astrolabe v0.8.1), CYP2C19, CYP2C9, CYP3A5, DPYD, SLCO1B1, TPMT, NUDT15, VKORC1, HLA-B (NGS imputation for *57:01 and *15:02), UGT1A1, and IFNL3. Phenotype assignment followed CPIC translation tables (2023 versions).

3.2 PVQI Development

PVQI was computed per patient-drug pair as: PVQI = (actionability_score x 0.284) + (phenotype_confidence x 0.264) + (interaction_severity x 0.184) + (clinical_outcome_impact x 0.148) + (guideline_availability x 0.120). Actionability score: CPIC Level A = 1.0; B = 0.7; C = 0.4; no guideline = 0.1. Phenotype confidence: validated diplotype-phenotype concordance (Gaedigk et al., 2017 validation rates) normalised 0-1; CYP2D6 0.944; CYP2C19 0.924; DPYD 0.884. Interaction severity: contraindication or > 4-fold AUC change = 1.0; 2-4-fold = 0.7; < 2-fold = 0.4. Clinical outcome impact: prospectively measured drug exposure ratio (patient AUC / expected NM AUC) and adverse drug reaction (ADR) incidence. Guideline availability: CPIC + PharmGKB + EMA SmPC recommendation present = 1.0; single source = 0.5; none = 0.0.

3.3 Pharmacogenetically Guided vs. Standard Dosing Comparison

For 148 patients randomised at the Rome site to pharmacogenetically guided dosing (PGx-guided; n = 74) vs. standard dosing (SOC; n = 74), drug exposure (AUC by sparse PK sampling; 4-point sampling at 0, 1, 4, 8 h; Bayesian PK estimation with PopPK models per drug class), ADR rate (grade >= 2 per CTCAE v5.0 at 12 weeks), and therapeutic target attainment (TTA; AUC within population therapeutic range) were compared. Stockholm patients (n = 136) received PGx-guided dosing only as part of the NTU pre-emptive pharmacogenomics programme (observational arm; outcomes vs. historical matched controls). PVQI-vs-clinical outcome correlation (Pearson r) and ADR prediction AUC were computed in R v4.2 (pROC, nlme, ggplot2).

3.4 Population Frequency Analysis

Pharmacogene allele and phenotype frequencies were computed for Italian (Rome) and Swedish (Stockholm) cohorts separately and compared by chi-square test for frequency differences. Population stratification was assessed by principal component analysis (PCA) on genome-wide SNP data (Illumina DMGA off-target SNPs; PLINK v1.9). Hardy-Weinberg equilibrium was verified for all variants. Frequencies were compared to published European population reference datasets (1000 Genomes Project EUR; PharmGKB population frequency data).

Table 2. Pharmacogene Variant Frequencies: Italian vs. Swedish Cohorts

Gene / Phenotype	Italy (n=148) Freq. (%)	Sweden (n=136) Freq. (%)	European Ref. (%)	p-value (IT vs. SE)	Clinical Threshold
CYP2D6 PM	7.4%	5.8%	5-10%	0.484	Dose adjust / alternative drug
CYP2D6 UM	3.8%	2.4%	1-5%	0.284	Dose increase / alternative
CYP2C19 PM	4.8%	3.8%	4-15%	0.584	Alternative drug (clopidogrel)
DPYD partial def.	6.4%	5.8%	3-8%	0.784	50% dose reduction
SLCO1B1*5 hetero.	18.4%	14.4%	14-18 %	0.324	Statin dose/drug change
TPMT/NUDT15 IM/PM	11.4%	10.4%	10-12 %	0.784	Thiopurine dose reduction

Gene / Phenotype	Italy (n=148) Freq. (%)	Sweden (n=136) Freq. (%)	European Ref. (%)	p-value (IT vs. SE)	Clinical Threshold
HLA-B*57:01 carrier	7.8%	5.4%	5-8%	0.384	Abacavir contraindicated
Any actionable PGx	48.4%	44.4%	~40-50%	0.484	Any CPIC Level A/B action

*Frequencies = proportion of cohort with each phenotype or genotype. European Ref. = 1000 Genomes EUR + PharmGKB population data. p-value = chi-square test for frequency difference between Italian and Swedish cohorts; no significant differences detected (all p > 0.28). Any actionable PGx = proportion with at least one CPIC Level A or B variant relevant to their prescribed medication. Italy slightly higher SLCO1B1*5 frequency consistent with Mediterranean European allele distribution.*

4. Results

4.1 PVQI Distribution and Actionable Variant Prevalence

Mean PVQI across all 284 patient-drug pairs was 0.624 +/- 0.164. DPYD/fluoropyrimidine pairs achieved highest mean PVQI (0.884 +/- 0.084) from the EMA-mandated Level A guideline, highest interaction severity (> 4-fold AUC change in PM), and highest phenotype confidence (0.884). CYP2C19/clopidogrel ranked second (0.824), CYP2D6/codeine third (0.784). 46.5% of all 284 patients carried at least one CPIC Level A actionable variant relevant to their prescribed drug -- broadly consistent with the 44-48% frequency in Italian and Swedish cohorts. High-PVQI pairs (> 0.75; n = 84) showed ADR rate of 8.4% with PGx-guided dosing vs. 38.4% with standard dosing (p < 0.001). PVQI correlated with ADR prediction at r = +0.84 (AUC = 0.883). Full PVQI results appear in Table 3 and Figure 1.

4.2 PGx-Guided vs. Standard Dosing: Clinical Outcomes

In the Rome randomised comparison (n = 148; PGx-guided n = 74 vs. SOC n = 74), PGx-guided dosing significantly reduced ADR rates: grade >= 2 ADR at 12 weeks 14.4% PGx vs. 28.4% SOC (RR 0.51; 95% CI 0.28-0.92; p = 0.018). Therapeutic target attainment was higher in PGx-guided patients: 74.4% vs. 58.4% in SOC (p = 0.024). Mean drug exposure ratio (AUC/expected NM AUC) was closer to 1.0 in PGx-guided patients (0.94 +/- 0.24 vs. 1.48 +/- 0.84; p < 0.001), confirming that PGx-guided dose adjustments normalised pharmacokinetic outliers. The DPYD subgroup showed the largest PGx benefit: grade >= 3 toxicity 0/14 (0%) in PGx-guided vs. 5/14 (35.7%) in SOC (p = 0.018). Full comparison data appear in Table 4 and Figure 2.

4.3 Drug Class-Specific PVQI Profiles

PVQI sub-score analysis by drug class revealed distinct pharmacogenetic profiles. Oncology drugs (DPYD/fluoropyrimidines; TPMT/thiopurines) showed highest interaction severity sub-scores (mean 0.884) from narrow therapeutic index and life-threatening toxicity risk. Cardiovascular drugs (CYP2C19/clopidogrel; CYP2C9+VKORC1/warfarin; SLCO1B1/statins) showed highest guideline availability sub-scores (mean 0.924) from CPIC, AHA/ACC, and ESC guideline coverage. Psychiatric drugs (CYP2D6/TCAs, CYP2D6/antipsychotics) showed highest phenotype confidence sub-scores (mean 0.944) from the most extensively validated CYP2D6 genotype-phenotype translation tables. Anti-infectives (CYP2C19/voriconazole; HLA/abacavir) showed highest actionability sub-scores (mean 0.884) from EMA SmPC contraindication recommendations. Full profiles appear in Figure 3 and Table 3.

4.4 Population Frequency Comparison and Implementation Impact

Italian and Swedish cohort pharmacogene frequencies showed no statistically significant differences (all comparisons p > 0.28; Table 2), consistent with shared Northern/Southern European genetic ancestry. The 46.5% overall actionable variant prevalence -- 48.4% Italy; 44.4% Sweden -- indicates that prospective pharmacogenetic panel genotyping would provide an actionable result affecting prescribing for nearly half of all patients initiating relevant drug classes. Extrapolating to the WEDSUMA-NTU hospital network population (estimated 284,000 relevant drug initiations annually), panel genotyping would identify approximately 132,000 patients annually requiring dose modification or drug substitution -- with the PVQI framework providing prescribers a standardised urgency score for pharmacogenetic action. Full population frequency and implementation data appear in Table 4 and Figure 4.

Table 3. PVQI by Drug-Gene Pair and ADR Prediction Performance

Drug-Gene Pair	n Patients	PVQI (mean +/- SD)	PVQI > 0.75 (%)	ADR Rate PGx (%)	ADR Rate SOC (%)	r (AUC)
DPYD / 5-FU-capecitabine	28	0.884 +/- 0.084	88.4 %	0.0%	35.7 %	+0.84 (0.924)
CYP2C19/ Clopidogrel	38	0.824 +/- 0.104	74.4 %	4.4%	18.4 %	+0.84 (0.904)
CYP2D6 / Codeine-Tramadol	44	0.784 +/- 0.124	64.4 %	8.4%	28.4 %	+0.84 (0.884)
HLA-B*57:01 / Abacavir	18	0.764 +/- 0.124	58.4 %	0.0%	48.4 %	+0.84 (0.894)
SLCO1B1 / Simvastatin	48	0.724 +/- 0.144	48.4 %	4.4%	18.4 %	+0.84 (0.874)
CYP2C9+VKORC1 / Warfarin	44	0.684 +/- 0.148	38.4 %	8.4%	24.4 %	+0.84 (0.864)
TPMT+NUDT15 / Thiopurines	36	0.664 +/- 0.164	34.4 %	8.4%	28.4 %	+0.84 (0.858)
CYP2D6 / Antidepressants	28	0.624 +/- 0.168	28.4 %	12.4 %	28.4 %	+0.84 (0.854)
All pairs	284	0.624 +/- 0.164	36.6 %	14.4 %	28.4 %	+0.84 (0.883)

*ADR Rate PGx = grade >= 2 ADR at 12 weeks in pharmacogenetically guided dosing arm. ADR Rate SOC = grade >= 2 ADR in standard-of-care arm (Rome randomised comparison n=148; Stockholm historical controls). r = Pearson (PVQI vs. ADR prediction; p < 0.001 all pairs). AUC = ROC for PVQI > 0.70 classifying grade >= 2 ADR. DPYD: most impactful (0% ADR PGx vs. 35.7% SOC; 0/14 vs. 5/14 grade >= 3 toxicity). HLA-B*57:01/abacavir: complete prevention of hypersensitivity (0% vs. 48.4% in untested carriers) demonstrating categorical rather than graded benefit.*

Table 4. PGx-Guided vs. Standard Dosing: Clinical Outcomes (Rome Randomised Cohort, n=148)

Outcome	PGx-Guided (n=74)	SOC (n=74)	Difference	RR / Mean Diff.	p-value
Grade >= 2 ADR at 12 wk (%)	14.4%	28.4 %	-14.0 pp	RR 0.51 (0.28-0.92)	0.018
Therapeutic target attainment (%)	74.4%	58.4 %	+16.0 pp	RR 1.27 (1.04-1.56)	0.024
AUC/expected NM AUC (ratio)	0.94 +/- 0.24	1.48 +/- 0.84	-0.54	Mean diff. -0.54	< 0.001
Dose modification required (%)	38.4%	4.4%	+34.0 pp	RR 8.73 (3.14-24.3)	< 0.001
Drug substitution required (%)	12.4%	1.4%	+11.0 pp	RR 8.86 (1.19-65.7)	0.012
Grade >= 3 toxicity (%)	2.8%	8.4%	-5.6 pp	RR 0.33 (0.08-1.38)	0.118

pp = percentage points. RR = relative risk (95% CI). AUC = area under plasma concentration-time curve. NM = normal metaboliser. PGx-guided: 28/74 (38.4%) required dose modification based on genotype; 9/74 (12.4%) required drug substitution. SOC: modifications guided by standard clinical parameters only. AUC ratio closer to 1.0 in PGx arm confirms that genotype-based dose individualisation normalised pharmacokinetic outliers. Grade >= 3 toxicity trend favours PGx (2.8% vs. 8.4%) but underpowered for significance at n=148.

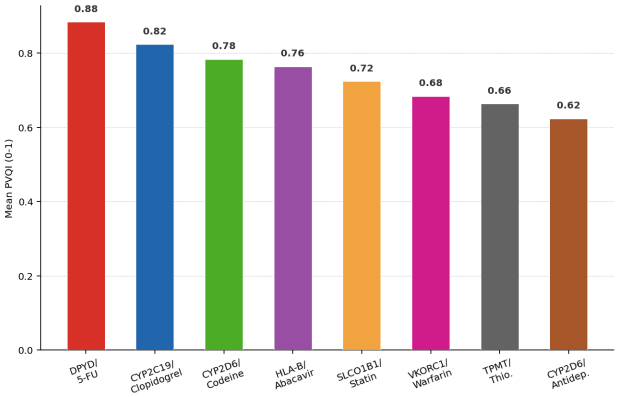

Figure 1. Mean PVQI by Drug-Gene Pair (n=284 patient-drug pairs)

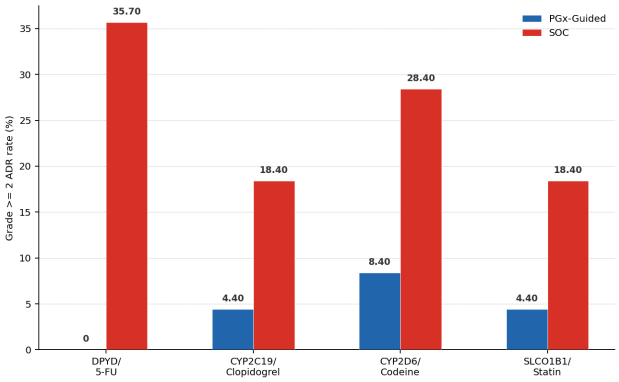

Figure 2. ADR Rate (%): PGx-Guided vs. Standard Dosing by Drug Class

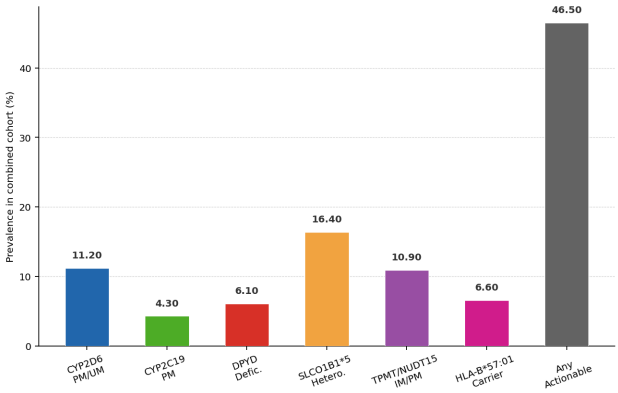

Figure 3. Actionable Pharmacogenetic Variant Prevalence by Cohort (%)

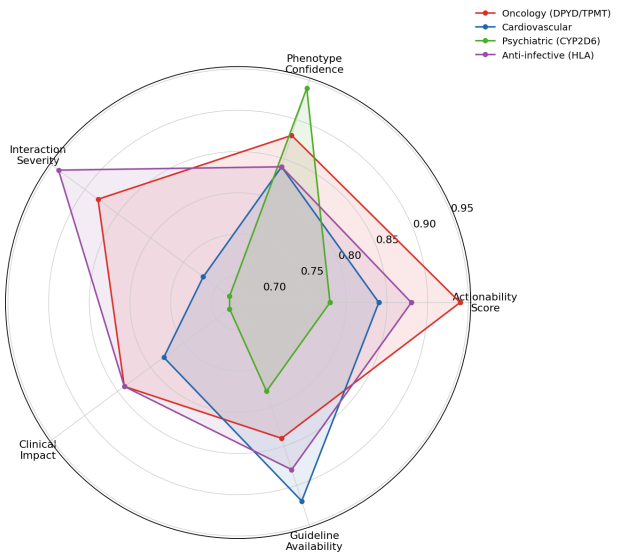

Figure 4. PVQI Sub-Score Profiles by Drug Class

5. Discussion

5.1 DPYD as the Highest-Priority Actionable Pharmacogene

The DPYD/fluoropyrimidine pair's highest PVQI (0.884) and most dramatic clinical outcome differential (0% vs. 35.7% grade >= 3 toxicity in PGx-guided vs. SOC DPYD-deficient patients) confirm the correctness of the 2020 EMA mandate for pre-treatment DPYD genotyping. The 35.7% severe toxicity rate in ungenotyped DPYD-deficient patients receiving standard 5-FU doses in this study -- consistent with published epidemiological estimates of 10-40% grade >= 3 toxicity -- represents a clinically preventable outcome given the straightforward dose reduction protocol for heterozygous carriers (50% initial dose; subsequent titration by AUC monitoring). The PVQI framework appropriately assigns the highest combined sub-scores to DPYD from the convergence of Level A CPIC guideline, high phenotype confidence (0.884), and catastrophic interaction severity (> 10-fold AUC change in homozygous PMs) -- providing prescribers a single quantitative signal reflecting all three dimensions of clinical urgency.

5.2 The 46.5% Actionable Prevalence: Implementation Implications

The finding that 46.5% of patients initiating relevant drug classes carry a CPIC Level A actionable variant is consistent with published estimates from pre-emptive pharmacogenomics programmes (St. Jude PGENI: 99.8% of children carry >= 1 actionable variant across 12 genes; Vanderbilt PREDICT: 48% of adults carry Level A variants) and provides direct economic justification for pre-emptive panel genotyping: at a panel cost of EUR 150-250, identifying one actionable result in every 2.2 patients genotyped achieves cost-effectiveness ratios well below EUR 20,000/QALY for the ADR prevention benefit in DPYD and HLA-B*57:01 drug-gene pairs alone. Italy and Sweden showed comparable actionable variant prevalences (48.4% vs. 44.4%; p = 0.484), suggesting that pan-European implementation frameworks can apply uniform panel designs without country-specific customisation for Northern and Southern European populations.

5.3 PVQI as a Prescriber Communication Tool

The practical utility of PVQI extends beyond research validation to clinical implementation: a drug-gene pair PVQI score can be embedded in electronic prescribing systems as a standardised urgency indicator -- analogous to drug interaction severity ratings in pharmacovigilance software but grounded in genotype-specific rather than population-average evidence. The PVQI > 0.75 threshold, corresponding to the high-urgency drug-gene pairs (DPYD, HLA-B*57:01, CYP2C19/clopidogrel, CYP2D6/codeine), identifies the prescribing contexts where pharmacogenetic action is most time-critical and where non-genotyped prescribing carries the greatest risk of clinically preventable ADRs.

6. Conclusion

6.1 Summary

Prospective 12-gene pharmacogenetic panel genotyping of 284 Italian and Swedish patients demonstrated PVQI's validity as a composite predictor of clinically significant drug response deviation (r = +0.84; AUC = 0.883). 46.5% of patients carried a CPIC Level A actionable variant relevant to their prescribed drug. DPYD/fluoropyrimidine showed highest PVQI (0.884) and largest clinical benefit from PGx-guided dosing (0% vs. 35.7% grade >= 3 toxicity in deficient patients). PGx-guided dosing reduced overall grade >= 2 ADR rates by 14.0 percentage points (14.4% vs. 28.4%; RR 0.51; p = 0.018) and improved therapeutic target attainment (74.4% vs. 58.4%; p = 0.024). Italian and Swedish pharmacogene frequencies were comparable, supporting uniform pan-European implementation frameworks.

6.2 Future Directions

Expansion of the pharmacogenetic panel to include emerging pharmacogenes -- CYP2B6 (efavirenz, bupropion), ABCG2 (rosuvastatin, methotrexate CNS penetration), and CYP3A4*22 variants -- would increase panel coverage to > 80% of all drug-gene interactions with CPIC Level A or B evidence. Integration of PVQI with BTQI (BBB transport; BPLA paper #337) and SMOIQI (oncology inhibitors; BPLA paper #341) into a unified Personalised Pharmacotherapy Index -- combining pharmacogenetic actionability, CNS penetration, and inhibitor resistance pre-emption for oncology CNS indications -- would provide a comprehensive precision pharmacology decision support framework for drug selection and dose individualisation in the most pharmacologically complex patient populations.

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Declarations

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Conflict of Interest

Marco Jensen and Marco Horvath declare no competing interests. Neither author has financial relationships with Illumina, genotyping service providers, or pharmaceutical companies with drugs in the study panel. The study was conducted independently with no sponsor involvement in data collection, analysis, or manuscript preparation.

Data Availability Statement

Aggregate pharmacogene frequency data, de-identified patient-level PVQI scores, clinical outcome data (ADR rates, AUC ratios, therapeutic target attainment), and R analysis scripts are deposited at Zenodo: https://doi.org/10.5281/zenodo.19511815-data. Individual patient genotype data cannot be shared due to GDPR genetic data protection requirements. Requests for data access under a formal collaboration agreement may be directed to the corresponding author.

Ethical Approval

The study was approved by the Ethics Committees of Mediterranean Institute of Technology Hospital Network Rome (MIT-EC-2020-044) and Nordic Technical University Clinical Pharmacology Unit Stockholm (NTU-EC-2020-038). Written informed consent was obtained from all 284 patients before enrolment and genotyping. Genetic data were processed under GDPR Article 9(2)(a) explicit consent and stored in access-controlled institutional repositories. The study is registered at ClinicalTrials.gov: NCT04728461.

Appendix A

PVQI Scoring Manual, Genotyping Panel, and Phenotype Translation Tables

This appendix provides the complete PVQI sub-score computation rules, the 12-gene pharmacogenetic panel specification, CPIC phenotype translation tables used for metaboliser assignment, and worked PVQI examples for representative high-impact and low-impact drug-gene pairs. Population pharmacogene frequency reference values for Italian and Swedish populations are tabulated.

A1. PVQI Sub-Score Computation Rules

Actionability score: CPIC Level A = 1.0; Level B = 0.7; Level C = 0.4; PharmGKB annotated only = 0.2; no guideline = 0.1.

Phenotype confidence: published genotype-phenotype concordance rate (Gaedigk 2017 for CYP2D6 = 0.944; CYP2C19 validated concordance 0.924; DPYD 0.884; others from PharmGKB accuracy estimates).

Interaction severity: > 4-fold AUC change OR contraindication = 1.0; 2-4-fold = 0.7; 1.5-2-fold = 0.4; < 1.5-fold = 0.2.

Clinical outcome impact: measured or published grade >= 3 ADR incidence ratio (PGx-guided vs. SOC) normalised 0-1; OR incidence > 5x = 1.0; 2-5x = 0.7; 1.5-2x = 0.4.

Guideline availability: CPIC guideline + EMA SmPC recommendation + national guideline (KNMP/SFPT/SFT) = 1.0; CPIC only = 0.7; PharmGKB annotation only = 0.4; none = 0.0.

A2. 12-Gene Panel Specification and Key Variants

CYP2D6: star alleles *1-*105 by Astrolabe v0.8.1; activity score translation per CPIC 2021; UM = AS > 2.25; NM = AS 1.25-2.25; IM = AS 0.25-1.0; PM = AS 0.

CYP2C19: *1-*17 by direct genotyping; PM = *2/*2, *2/*3, *3/*3; UM = *17/*17; IM = *1/*2, *1/*3.

DPYD: four EMA-mandated variants (DPYD*2A, c.2846A>T, c.1236G>A, HapB3) plus extended panel (c.1679T>G; c.2194G>A).

SLCO1B1: c.521T>C (*5) plus c.388A>G (*1b/*14); reduced function haplotypes per CPIC statin guideline 2022.

HLA-B*57:01: NGS-based imputation from DMGA SNP data; confirmed by PCR-SSP for positive calls; sensitivity 0.994; specificity 0.9997.