Fundamental & Clinical Pharmacology

WILEY | Fundamental & Clinical Pharmacology SFPT

REVIEW ARTICLE

French-Speaking Network of Pharmacogenetics (RNPGx) Recommendations for Gene Panel Analysis Through Genotyping or Sequencing in Pharmacogenetics

Nicolas Picard[1] | Estelle Ayme-Dietrich[2] | Sylvie Quaranta[3] | Tiphaine Adam-De-Beaumais[4] | Abd El Kader Ait Tayeb[5,6] | Hugo Alarcan[7] | Dorra Amor[8] | David Barthelemy[9] | Mouna Ben Sassi[10,11] | Séverine Cunat[12] | Mouna Daldoul[10,11] | Hervé Delacour[13] | Marie-Christine Etienne-Grimaldi[14] | Xavier Fonrose[15] | Benjamin Hennart[16] | Louis Lebreton[17] | Stephanie Malard[18] | Céline Narjoz[19] | Asma Omezzine[8] | Nicolas Pallet[19] | Léa Payen[9] | Jeanne Petit[20] | Fabienne Thomas[20] | Sameh Trabelsi[10,11] | Camille Tron[21] | Céline Verstuyft[5,22] | Paul Vilquin[23] | Jean-Christophe Boyer[24] | Vincent Haufroid[25,26] | French-Speaking Network of Pharmacogenetics (RNPGx)

[1]Department of Pharmacology, Toxicology and Pharmacovigilance, Inserm 1248 P&T, CHU Limoges, Limoges, France | [2]Laboratory of Neurocardiovascular Pharmacology and Toxicology, UR7296, University Hospitals of Strasbourg, Strasbourg, France | [3]Laboratory of Molecular Biology GEnOPé / Laboratory of Pharmacokinetic and Toxicology, Timone Hospital, AP-HM, Marseille, France | [4]Department of Children and Adolescents Oncology, Gustave Roussy, Villejuif, France | [5]Department of Genomic and Pharmacogenomic Medicine, Assistance Publique-Hôpitaux de Paris, Paris-Saclay University Hospitals, Bicêtre Hospital, Le Kremlin Bicêtre, France | [6]Inserm, Endocrine Physiology and Physiopathology, Paris-Saclay University, Le Kremlin-Bicêtre, France | [7]Department of Biochemistry and Molecular Biology, CHRU Tours, Tours, France | [8]Faculty of Pharmacy, LR 12Sp11, Department of Biochemistry, CHU Sahloul Sousse, Monastir University, Monastir, Tunisia | [9]Multi-site Laboratory of Medical Biology and Pathology of the University Hospital of Lyon (LBMMS), Department of Biochemistry and Molecular Biology, Medical Unit of Pharmacology, Pharmacotoxicology and Pharmacogenetics (PPTO) Lyon-Sud Hospital, Hospices Civils de Lyon, Pierre-Bénite, France | [10]Clinical Pharmacology Department, Chalbi Belkahia National Center of Pharmacovigilance, Tunis, Tunisia | [11]Laboratory of Research in Clinical and Experimental Pharmacology LR16SP02, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia | [12]Department of Biological Hematology, Montpellier University Hospital, Montpellier, France | [13]Department of Biology, National Military Teaching Hospital BEGIN, Saint Mandé, France | [14]Oncopharmacology Department, and Laboratory of Translational Research in Oncology (LRTO), Antoine Lacassagne Center, Côte d'Azur University, Nice, France | [15]Laboratory of Pharmacology, Pharmacogenetics and Toxicology, Grenoble Alpes University Hospital, Grenoble, France | [16]Department of Toxicology and Genopathies, CHU Lille, Lille, France | [17]Laboratory of Biochemistry, University Hospital Centre Bordeaux, Bordeaux, France | [18]Cerba Laboratory, Frepillon, France | [19]Biochemistry Department, Georges Pompidou European Hospital, Assistance Publique-Hôpitaux de Paris, Paris Cité University, Paris, France | [20]Pharmacology Department, Oncopole Claudius Regaud, IUCT-Oncopole and Toulouse Cancer Research Center (CRCT), Toulouse University, Toulouse, France | [21]Inserm, EHESP, Irset (Institute for Research in Health, Environment and Work) – UMRS 1085, Rennes University, CHU Rennes, Rennes, France | [22]MOODS Team, INSERM CESP, Paris-Saclay Faculty of Medicine, Paris-Saclay University Hospitals, Bicêtre Hospital, Le Kremlin Bicêtre, France | [23]Department of Tumor Genomics and Pharmacology, Paris-Cité University, Inserm UMRS 976, Saint-Louis Hospital, AP-HP Paris, Paris, France | [24]Laboratory of Biochemistry and Molecular Biology, CHU Carémeau, Nimes, France | [25]Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Institut de Recherche Expérimentale et Clinique (IREC), UCLouvain, Brussels, Belgium | [26]Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Brussels, Belgium

Correspondence: Sylvie Quaranta (sylvie.quaranta@ap-hm.fr)

Received: 23 June 2025 | **Revised:** 13 November 2025 | **Accepted:** 2 December 2025

Keywords: clinical implementation | genotyping | next-generation sequencing | pharmacogenetics

ABSTRACT

Background: The implementation of pharmacogenetics in clinical practice increasingly relies on multigene panels.

Objectives: The objective of this study is to develop harmonized recommendations for the design and analytical implementation of multigene pharmacogenetic panels, defining clinically relevant genes and associated regions of interest (ROIs) based on evidence strength, therapeutic applicability, and compatibility with genotyping or sequencing technologies.

Methods: The French-Speaking Network of Pharmacogenetics (RNPGx) evaluated 81 candidate genes across five therapeutic domains (i.e., oncology and supportive care, anesthesia and pain management, cardiology, neurology and psychiatry and immunology and infectious diseases) using a structured, evidence-based scoring system. Each gene was evaluated using a 25-point scoring system integrating pharmacogenetic importance, regulatory and professional society recommendations, and expert consensus. For the genes ultimately selected for the core panel, clinically relevant regions of interest were defined and assigned to one of three analytical classes. Class 1 includes variants with established clinical actionability; Class 2 adds optional variants suitable for extended testing in specialized settings; and Class 3 covers broader genomic regions mainly intended for rare variant or structural analyses.

Results: A 28-gene core panel was retained. Class 1 included 76 prioritized variants (including CYP2D6 CNV variants), and Class 2 comprised 62 additional variants (with extended analysis for CYP2D6). Class 3 eligibility was retained for 18 genes.

Conclusion: The RNPGx recommendations offer a harmonized and flexible framework for pharmacogenetic panel design and for the extraction and interpretation of pharmacogenetic data from whole-exome or whole-genome sequencing.

1 | Introduction

Pharmacogenetics (PGx) aims to optimize drug therapy by using patients' genetic profiles to guide individualized drug and dose selection. Given that nearly 99% of the population carries at least one actionable PGx variant and that 60.4% of individuals in the general population are likely to receive at least one pharmacogenetically actionable drug [1], PGx panel testing is increasingly recognized as a promising strategy for enhancing drug safety and efficacy [2]. This recognition is supported by robust evidence from multiple studies, including randomized controlled trials, demonstrating that individualizing drug therapy based on pharmacogenetic profiling leads to improved patient outcomes [3–5]. Specifically, a recent real-world implementation study conducted across seven European countries involving 6944 patients demonstrated that genotype-guided prescribing using a 12-gene pharmacogenetic panel significantly reduced the incidence of clinically relevant adverse drug reactions [3]. Although the study has faced methodological criticisms, mainly related to its open-label crossover design, endpoint selection, and outcome interpretation, Guchelaar et al. [6] provided a balanced review of these issues, turning them into practical lessons for future pharmacogenetic implementation research. Compared to targeted genotyping, which identifies key variants relevant to specific drug-gene pairs, gene panels provide a more comprehensive dataset, allowing for the selection of alternative therapies and anticipating responses to additional drugs beyond the initial clinical indication. This broader perspective is particularly valuable when PGx data are obtained from whole exome or whole genome sequencing (WES and WGS) performed as part of the diagnostic of the primary disease, as increasingly seen with the use of next-generation sequencing (NGS) in clinical care. Two main types of panel-based testing are currently employed: (1) disease-oriented panels, which combine genes relevant to drugs commonly used in a particular therapeutic area, and (2) large pretherapeutic panels, which include a wide array of clinically actionable variants related to multiple clinical contexts.

This categorization has been reviewed in detail by Mosch et al. [2], highlighting the rationale, opportunities, and limitations behind each approach. The advent of second-generation sequencing favored the second approach by enabling the simultaneous analysis of multiple genes.

The content of PGx panels and the interpretation of their results are facilitated by the existence of guidelines issued by consortia such as the Clinical Pharmacogenetics Implementation Consortium (CPIC), the Dutch Pharmacogenetics Working Group (DPWG), the Canadian Pharmacogenomics Network for Drug Safety (CPNDS), and the French-Speaking Network of Pharmacogenetics (RNPGx). Additionally, regulatory agencies such as the European Medicines Agency (EMA), US Food and Drug Administration (FDA), SwissMedic, or Health Care Service Corporation (HCSC) influence the design and application of PGx panels through their drug labeling and safety communications, which can differ from one country to another. ClinPGx serves as a comprehensive knowledge base that compiles these guidelines and recommendations. However, obtaining a global overview of which genes to include in PGx panels remains challenging due to some divergences in recommendations between different consortia and regulatory agencies. These discrepancies can be attributed to differences in the interpretation of evidence levels, population-specific allele frequencies, and clinical implementation strategies.

To address these gaps and support clinical application of PGx, including the reanalysis of WES/WGS data generated as part of patient care, the RNPGx conducted a comprehensive selection of genes based on the latest evidence available. For each core gene, regions of interest were identified to target using low-, medium-, and high-throughput analytical strategies. The aim of this work was to establish a validated list of genes that can serve as a reference for the design and implementation of pharmacogenetic panels. To reflect differences in clinical utility and context of testing, the recommendations distinguish three classes of variants or genomic regions.

Class 1 variants correspond to the minimal set of pharmacogenetic markers with well-established clinical actionability; they are supported by strong evidence and international guidelines and can be used directly in routine clinical practice, possibly in preemptive settings. Class 2 variants remain clinically relevant but represent an optional extension of the Class 1 content; they may be considered in selected clinical situations where a more comprehensive analysis is warranted, such as unexplained drug response or severe adverse reactions. Class 3 regions include broader genomic areas (exons, untranslated regions, and promoter sequences). Their inclusion primarily serves to enable

the detection of rare variants that may refine metabolizer phenotype prediction and to explore genes that may harbor copy number variations, structural variants, or multiple co-occurring variants. The recommendations are organized into five major therapeutic domains: oncology and supportive care, anesthesia and pain management, cardiology, neurology and psychiatry, and immunology and infectious diseases. These guidelines are intended to provide valuable insights for professionals involved in PGx and to serve as a resource for biotechnology or decision-support companies developing solutions tailored to clinical needs.

2 | Methods

2.1 | RNPGx Gene Selection Process

The RNPGx working group employed a scoring system to identify and prioritize genes for inclusion in the pharmacogenetic panel. The initial selection of genes for evaluation came from two sources: on the one hand, 41 genes derived from the *Pharmacogenomics Community Panel* developed by SOPHiA GENETICS in collaboration with RNPGx (i.e., *ABCB1*, *ABCC2*, *ABCC4*, *ABCG2*, *ACE*, *ADRB1*, *ADRB2*, *BCHE*, *CDA*, *COMT*, *CYP1A2*, *CYP2B6*, *CYP2C19*, *CYP2C8*, *CYP2C9*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *CYP4F2*, *DPYD*, *DRD2*, *G6PD*, *HTR2A*, *ITPA*, *MTHFR*, *NAT2*, *NR1I2*, *NR1I3*, *NUDT15*, *OPRM1*, *P2RY12*, *POR*, *RYR1*, *SLC22A1*, *SLCO1B1*, *TPMT*, *TYMS*, *UGT1A1*, *UGT1A4*, *UGT2B7*, and *VKORC1*) and, on the other hand, 40 additional genes identified during a preliminary open consultation with RNPGx members regarding five clinical situations: oncology and supportive care, anesthesia and pain management, cardiology, neurology and psychiatry, and immunology and infectious diseases (i.e., *ALDH5A1*, *CACNA1S*, *CDKL5*, *CEP72*, *CES1*, *CES2*, *CNR1*, *CPS-1*, *CTH*, *CYP1A1*, *CYP2A6*, *CYP2C cluster*, *CYP2E1*, *ERCC1*, *ERCC2*, *GGCX*, *GSTM1*, *GSTP1*, *HLA-A*, *HLA-B*, *HTR1C*, *HTR2B*, *IMPDH1*, *IMPDH2*, *INFL4*, *KCNJ6*, *MT-RNR1*, *OTC*, *PEAR1*, *POLG*, *PON1*, *RARG*, *SLC22A2*, *SLC28A3*, *SLC6A4*, *SLCO1B3*, *TRPV1*, *UGT1A6*, *UGT2B4*, and *XRCC1*).

The gene selection process followed a structured, multistep methodology involving domain-specific expertise and multiple levels of validation. The RNPGx working group was divided into five subgroups, each focusing on the specific pharmacological domain mentioned above. Each subgroup was led by a coordinator with expertise in the field, who oversaw the scoring and decision-making process. The experts within each subgroup assigned scores to genes based on the predefined scoring system on a total score of 25 points, distributed across four criteria:

1. Very important pharmacogene (VIP) status (0–7.5 points): Genes were classified according to their pharmacogenetic importance as nonranked (0 points), Tier 2 (2.5 points), or Tier 1 (7.5 points) using the VIP classification system from ClinPGx.org.
2. Regulatory agency recommendations (0–7.5 points): We used ClinPGx website which consolidates recommendations from multiple agencies, including the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), Swissmedic, and Health Canada–Santé Canada (HCSC). Genes were scored based on the highest level of annotation provided by regulatory agencies in the clinical contexts considered, as detailed on ClinPGx.org. Scores were assigned as follows: 7.5 points for genes annotated at least once as "testing required"; 5 points for "testing recommended"; 2.5 points for "actionable PGx"; and 1 point for "informative PGx".
3. Scientific society recommendations (0–7.5 points): The number of endorsements from professional societies was quantified. Each recommendation contributed 2.5 points (up to a maximum of 7.5 points). Recommendations were primarily derived from CPIC, DPWG and RNPGx, but other expert groups indexed in ClinPGx were also taken into consideration when applicable.
4. RNPGx expert opinion (0–2.5 points): Experts provided qualitative evaluations, classifying genes as "to exclude" (0 point), "to consider" (1 point), or "to include" (2.5 points).

Genes with a score of ≥ 12.5 were automatically included in the preliminary core panel list, whereas those with a score of zero were excluded. Genes scoring above zero but below 12.5 were assigned to a secondary list.

The coordinator of each subgroup reviewed all assignments, either validating the decisions or suggesting further discussion. Independent reviewers, who did not participate in the scoring process, provided additional evaluations of the subgroup's choices. In cases of disagreement, decisions were made collegially through discussions within a steering group composed of the subgroup coordinators and the independent reviewers.

The final proposal of the steering group for classifying genes in the final core panel, including the discussion of genes with borderline scores, was circulated to all members of the working group for review and comments over a feedback period and was subsequently adopted by consensus during the RNPGx plenary assembly held in June 2024.

2.2 | RNPGx Region-of-Interest Selection Process

The selection of regions of interest (ROI) within the genes of the core panel selection prioritized clinical relevance while ensuring adaptability to different analytical strategies. A hierarchical approach allowed for a gradation of targeted regions, ranging from precise hot-spot variants (Class 1 or 2) to broader genomic ROIs indicated for a more extensive exploration in case of negative results for Class 1 or 2 or for research approaches (Class 3). A decision tree was applied to distinguish between Classes 1 and 2 variants based on functionality and clinical recommendation levels (Figure 1).

2.2.1 | Class 1

This class corresponds to the minimal set of variants required for clinical analysis using low-throughput, routine hospital

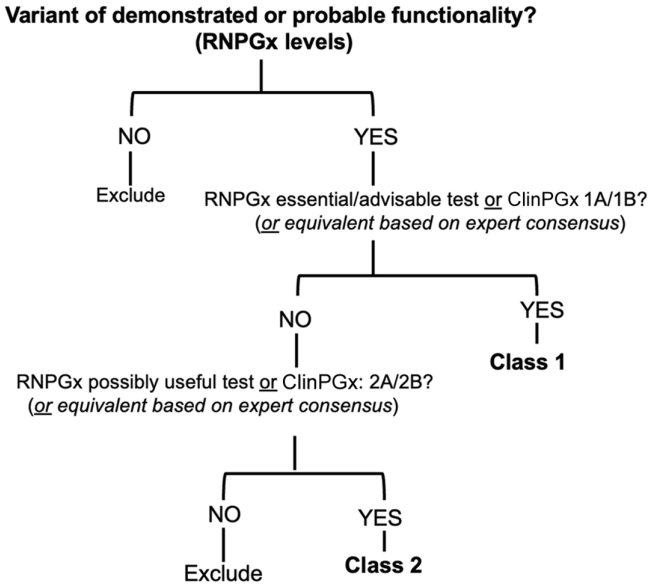

FIGURE 1 | Decision tree for classification of variants into Classes 1 and 2.

techniques, focusing on key pharmacogenetic insights essential for direct patient care. Only variants corresponding to the two highest levels of functional evidence as defined by the RNPGx in 2017 were considered (i.e., "demonstrated functionality" or "probable functionality") [7]. In addition, variants in this class must be associated either with the highest RNPGx level of recommendation for testing (i.e., essential or advisable tests) or with a Level 1A or 1B clinical annotation in the ClinPGx database. For variants lacking annotations from ClinPGx or RNPGx, the classification was determined by the expert group responsible for this work, based on expert consensus.

2.2.2 | Class 2

This class is designed for medium-throughput technologies, allowing for broader analyses that include additional clinically relevant variants while remaining feasible for specialized laboratories. To maintain the functional relevance of selected variants, only variants with "demonstrated functionality" or "probable functionality" based on the RNPGx 2017 definitions were included [7]. Variants categorized as Class 2 must meet the RNPGx requirement of a "possibly useful" test [7] or be associated with a Level 2A or 2B clinical annotation in the ClinPGx database. Similarly, for variants without prior annotations, classification decisions relied on expert review. Class 2 variants are not intended for first-line testing but may be considered when Class 1 testing is inconclusive or in research settings.

2.2.3 | Class 3

Unlike Classes 1 and 2, which focus on individual variants, Class 3 targets broader genomic regions. These ROIs may include entire exons, untranslated regions (UTRs), or promoter regions, providing a more comprehensive genomic coverage. The precise extent of these regions may vary depending on the analytical platform and data availability. The aim of this class is to draw attention to the potential added value of extended exploration, particularly for detecting structural or complex variants that may not be captured by standard Class 1 or 2 analyses. Some complex pharmacogenes, such as CYP2B6, CYP2D6, and HLA, indeed require specific sequencing and analytical strategies due to the presence of highly homologous pseudogenes and structural variants. For such regions, long-read sequencing technologies can provide more accurate characterization, as illustrated for the CYP2D6 locus, which contains the CYP2D7 and CYP2D8 pseudogenes.

3 | Results

Of the 81 genes included in the screening process, eight were excluded from further consideration as their score reflected a complete lack of evidence supporting their inclusion (i.e., *CTH*, *HTR2B*, *IMPDH1*, *IMPDH2*, *NR1I3*, *SLC22A1*, *SLC22A2*, and *SLCO1B3*).

3.1 | Core Gene Panel

From the 73 remaining genes, 22 were automatically assigned to the core panel, reflecting their supportive evidence and pharmacogenetic relevance (i.e., *ABCG2*, *CACNA1S*, *CYP2B6*, *CYP2C19*, *CYP2C9*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *CYP4F2*, *DPYD*, *G6PD*, *HLA-A*, *HLA-B*, *MT-RNR1*, *MTHFR*, *NUDT15*, *RYR1*, *SLCO1B1*, *TPMT*, *TYMS*, *UGT1A1*, and *VKORC1*; Table S1).

Among the 51 genes initially assigned to the secondary list, six genes were reassigned to the core selection after thorough discussion (i.e., *ABCB1*, *BCHE*, *CYP2C* cluster, *GGCX*, *NAT2*, and *POR*). The reassignment was based on emerging evidence, specific clinical contexts, and the consensus of the RNPGx working group. Specifically, the reassignment of *ABCB1* was justified by its potential clinical importance, as highlighted in a case report on Ivermectin [8]. The study described severe ivermectin toxicity linked to nonsense mutations in *ABCB1*, demonstrating the relevance of rare variants in this gene. The inclusion of the *BCHE* gene in the panel appeared justified by its critical role in diagnosing plasma cholinesterase deficiency detected through activity measurements in the context of prolonged neuromuscular block following the administration of curare. The *CYP2C* cluster region (i.e., rs12777823 and CYP2C:TG haplotype) was reassigned to the core panel due to its endorsement by CPIC in warfarin guidelines [9] for the first one and the phenotype impact of the CYP2C19 substrate for the second one. The *GGCX* (Gamma-glutamyl carboxylase) gene was also reassigned in the core selection due to its integration into warfarin dosing models [10].

The inclusion of *NAT2* was justified by a ClinPGx variant-drug annotation at Level 1B (regarding isoniazid) and its historical importance in PGx. A recent systematic review and meta-analysis

study further highlighted its critical role in predicting hepatotoxicity risk during tuberculosis treatment [11]. Finally, the *POR* gene was reassigned in the core panel due to its potential clinical relevance in optimizing tacrolimus dosing [12] and because only one variant is required for the pharmacogenetic analysis of this gene, which simplifies its inclusion and application in clinical algorithms.

Table 1 summarizes the 28 core panel genes selected by RNPGx as well as the clinical situations and drugs to which each gene is linked. Additional details, including gene-specific characteristics (e.g., chromosomal location, transcripts, exon count, and RNPGx scoring details), are provided in Table S1. In addition, Figure 2 provides another overview of the clinical domains in which their analysis is recommended. For each gene of the core panel, the recommended ROIs for Classes 1 and 2 analysis and eligibility for Class 3 analysis are summarized in Table 2.

3.1.1 | *ABCB1*

No Class 1 or 2 variants were retained for this gene (Table 2). Most studies investigating the associations between ***ABCB1*** genetic variants and drug effects have often produced inconsistent results. A key reason for this inconsistency is that the most extensively studied variants, such as *c.1236C > T*, *c.2677G > T/A*, and *c.3435C > T*, are highly prevalent in the general population, complicating the establishment of robust genotype–phenotype correlations. Sequencing overcomes the limitations of focusing exclusively on common variants.

As a result, **RNPGx** advocates for CDS sequencing for ***ABCB1*** (Class 3) as a more effective approach, allowing for the identification of rare variants that may have significant pharmacological impacts but remain unidentified. This interest is illustrated by the reported case of ivermectin toxicity in the literature [8].

3.1.2 | *ABCG2*

Over 80 single nucleotide polymorphisms (SNPs) of the *ABCG2* gene have been identified in DNA samples of ethnically different origins. The most common are p.V12M (c.34G > A, rs2231137) and p.Q141K (c.421C > A, **rs2231142**), highly prevalent in East Asians (MAF ~ 17%–45% and 22%–34%, respectively) and with relatively low allele frequencies in Caucasians (MAF ~ 3%–10% and 6%–14%, respectively) and African-American populations (MAF ~ 2%–12% and 1%–17%, respectively). All other genetic variants have an allelic frequency of 1% or less [13]. Among the two SNPs, only the missense variant (rs2231142) is ranked as Class 1 because it has been shown to reduce protein expression (30%–40%) and transport activity in vitro [14], and it is associated with Level 1A variant-drug annotations for rosuvastatin and allopurinol. Because *ABCG2* is a physiologically important urate transporter, congenital dysfunction of ABCG2 could be a risk factor for gout or hyperuricemia. Other rare low or no function genetic variants of *ABCG2* were identified in a European cohort with hyperuricemia risk and should be researched in second intention, with large sequencing technology (i.e., Class 3) [15].

3.1.3 | *BCHE*

The gene encodes butyrylcholinesterase (BChE), an enzyme essential for the hydrolysis of neuromuscular blocking agents such as succinylcholine and mivacurium [16].

Among the most studied *BCHE* variants, the atypical variant (**rs1799807**, p.Asp70Gly, also known as A-variant) is associated with reduced catalytic efficiency. It is a rare variant: Data from the 1000 Genomes Project indicate a MAF of 1% in the global population, with occurrence of the variant only in Europeans (MAF = 2%) and Americans (MAF = 1%). The presence of the atypical variant in a homozygous state is associated with a significant decrease in BChE activity (i.e., 50%–70% reduction). As a result, this mutation, which is found in a linkage disequilibrium (> 0.9) with the Kalow variant, is the most frequently found in cases of prolonged neuromuscular block in Indo-European patients. The Kalow variant (**rs1803274** or K variant) has a MAF ranging from 12% to 19% in the general population (1000 Genomes Project). It has a limited impact on BChE activity (i.e., approximately −30% in a patient harboring this mutation in the homozygous state) and is not definitively considered deleterious. Compound heterozygote patients (e.g., individuals carrying both A and K variants) do not exhibit significantly higher risk of severe and prolonged paralysis. Although the two variants are not associated with PharmGKB-level 1 variant-drug interactions, they are classified as Class 1 by RNPGx because they fall into the "advisable test definition" for succinylcholine or mivacurium (Figure 1) [7]. Clinical data suggest that the presence of other rare *BCHE* variants further exacerbates the effects of common mutations, leading to significantly prolonged neuromuscular blockade in affected individuals [17, 18]. Given the availability of robust enzymatic assays for characterizing variants, RNPGx classifies the sequencing of the *BCHE* gene as a Class 3 recommendation.

3.1.4 | *CYP2B6*

Among the 49-star allele haplotypes defined by the Pharmacogene Variation Consortium (PharmVar) for the CYP2B6 gene, three variants are well known to experts, because of their allelic frequency in the general population and their established functionality: *CYP2B6*4* (**rs2279343**), *CYP2B6*9* (**rs3745274**), and *CYPB6*18* (**rs28399499**). The first allele is associated with an increased CYP2B6 activity (as for *CYP2B6**22, in Class 2) with a MAF ranging from 13% to 34% in the general population, including all ethnic group. The last two alleles are, respectively, classified as reduced (*9 and several other associated alleles; see below) and loss-of-function (*18) alleles. Although rs3745274 has a MAF ranging from 22% to 38% in the general population, including all ethnic groups, rs28399499 is mainly present in African populations with a MAF between 6% and 12% in this ethnic group. Interestingly, the very well described *CYP2B6*6* allele is defined by the simultaneous presence of rs3745274 and rs2279343 Class 1 variants and is associated with a global reduced CYP2B6 activity, as for *CYP2B6*9*. In addition to *CYP2B6*9* and **6*, rs3745274 is also present in several other *CYP2B6* alleles associated with reduced CYP2B6 activity (**7, *13, *19, *20, *26, *34, *36, *37, *38, *39, *40, *41, *42*, and **43*), which is why this variant is clearly classified as Class 1 variant by

TABLE 1 | Pharmacogenes from the core selection, along with their associated clinical situations where testing is recommended, and the related drugs (i.e., those subject to a least one ClinPGx Level 1 or 2 clinical annotation or for which testing is ranked essential, advisable or potentially useful by RNPGx). Additional details, including gene-specific characteristics (e.g., chromosomal location, transcripts, exon count, and RNPGx scoring details), are provided in the Supporting Information.

Gene	Protein nature	Associated clinical situation(s)	Related drug(s)
ABCB1	Transporter	Immunoinfectiology	Ivermectin
ABCG2	Transporter	Cardiology	Allopurinol; rosuvastatin
BCHE	Enzyme	Pain-anesthesia	Succinylcholine; mivacurium
CACNA1S	Other (calcium channel)	Pain-anesthesia	Desflurane; enflurane; halothane; isoflurane; methoxyflurane; sevoflurane; succinylcholine;
CYP2B6	Enzyme (Cytochrome P450)	Immunoinfectiology	Efavirenz; nevirapine
		Neuropsychiatry	Bupropion; methadone; sertraline
CYP2C cluster (rs12777823) and *TG haplotype* (rs2860840/rs11188059)	Enzyme (Cytochrome P450)	Cardiology Neuropsychiatry/miscellaneous	Warfarin Sertraline; escitalopram; omeprazole
CYP2C19	Enzyme (Cytochrome P450)	Immunoinfectiology	Voriconazole
		Neuropsychiatry	Amitriptyline; citalopram; clomipramine; doxepin; escitalopram; imipramine; sertraline; trimipramine
		Cardiology	Clopidogrel; mavacamten
		Miscellaneous	Dexlansoprazole; lansoprazole; omeprazole; pantoprazole; rabeprazole
CYP2C9	Enzyme (Cytochrome P450)	Cardiology	Acenocoumarol; fluvastatin; warfarin
		Neuropsychiatry	Phenytoin; siponimod
		Pain-anesthesia	Celecoxib; flurbiprofen; ibuprofen; lornoxicam; meloxicam; piroxicam; tenoxicam
CYP2D6	Enzyme (Cytochrome P450)	Cardiology	Flecainide; metoprolol; propafenone
		Neuropsychiatry	Amitriptyline; aripiprazole; atomoxetine; clomipramine; desipramine; doxepin; fluvoxamine; imipramine; mirtazapine; nortriptyline; paroxetine; risperidone; trimipramine; venlafaxine; vortioxetine; zuclopenthixol
		Pain-anesthesia	Codeine; hydrocodone; oxycodone; tramadol
		Oncology and supportive care	Ondansetron; tamoxifen; tropisetron

(Continues)

TABLE 1 | (Continued)

Gene	Protein nature	Associated clinical situation(s)	Related drug(s)
CYP3A4	Enzyme (Cytochrome P450)	Immunoinfectiology	Tacrolimus
		Cardiology	Mavacamten
		Neuropsychiatry	Fentanyl; quetiapine; aripriprazole; brexpiprazole
CYP3A5	Enzyme (Cytochrome P450)	Immunoinfectiology	Tacrolimus
		Cardiology	Atorvastatin; anti-Xa direct-acting oral anticoagulants
CYP4F2	Enzyme (Cytochrome P450)	Cardiology	Warfarin
DPYD	Enzyme	Oncology and supportive care	Capecitabine; fluorouracile; tegafur
G6PD	Enzyme	Cardiology	Aspirin; rasburicase
		Pain-anesthesia	Aspirin; methylene blue
		Immunoinfectiology	Dapsone; nalidixic acid; nitrofurantoïne; primaquine; sulfadiazine; sulfafurazole; sulfamethoxazole; sulfaquanidine; sulfasalazine
GGCX	Enzyme	Cardiology	Warfarin
HLA-A	Other (HLA Class I)	Neuropsychiatry	Carbamazepine
		Oncology	TEDOPI, tebentafusp
HLA-B	Other (HLA Class I)	Immunoinfectiology	Abacavir; dapsone; flucloxacillin; sulfamethoxazole/trimethoprim
		Neuropsychiatry	Carbamazepine; lamotrigine; oxcarbazepine; phenytoin
		Miscellaneous	Allopurinol
MT-RNR1	Other (Mitochondrial rRNA)	Immunoinfectiology	Aminoglycosides
MTHFR	Enzyme	Oncology and supportive care	Methotrexate (at high dose only)
NAT2	Enzyme	Immunoinfectiology	Isoniazid
NUDT15	Enzyme	Immunoinfectiology	Azathioprine
		Oncology and supportive care	Mercaptopurine
POR	Enzyme	Immunoinfectiology	Tacrolimus
RYR1	Other (Ryanodine Receptor)	Pain-anesthesia	Desflurane; enflurane; halothane; isoflurane; methoxyflurane; sevoflurane; succinylcholine
SLCO1B1	Transporter	Cardiology	Atorvastatin; fluvastatin; lovastatin; pitavastatin; pravastatin; rosuvastatin; simvastatin
TPMT	Enzyme	Immunoinfectiology	Azathioprine
		Oncology and supportive care	Mercaptopurine

(Continues)

TABLE 1 | (Continued)

Gene	Protein nature	Associated clinical situation(s)	Related drug(s)
TYMS	Enzyme	Oncology and supportive care	5-fluorouracil; methotrexate
UGT1A1	Enzyme (UDP-glucuronosyltransferase)	Immunoinfectiology	Atazanavir
		Oncology and supportive care	Irinotecan; govitecan; nilotinib; erlotinib; belinostat
VKORC1	Enzyme	Cardiology	Acenocoumarol; warfarin

RNPGx. These three Class 1 variants are associated with Level 1A variant-drug annotations and CPIC guidelines for efavirenz [19] and sertraline [20] and with Level 2A variant-drug annotations for nevirapine, bupropion, and methadone (PharmGKB website). The RNPGx classified *CYP2B6*4*, **9* (and related alleles among which *6), and **18* as Class 1 variants, whereas other loss-of-function alleles **8, *12, *13, *28*, and **38* may be investigated as a second-line approach (Class 2). Finally, rare CYP2B6 structural variants are identified as *CYP2B7:CYP2B6* and *CYP2B6:CYP2B7* hybrid genes and designated by PharmVar as *CYP2B6*29* and **30* alleles, respectively. Their detection could improve metabolizer phenotype prediction, in the general population. The gene is thus eligible for Class 3 analysis.

3.1.5 | *CYP2C* Cluster

The CYP2C cluster on Chromosome 10 is a SNP rs12777823, identified through a GWAS in African American patients treated with warfarin [21].

RNPGx classifies this SNP as Class 2 due to its significant impact on warfarin dose, independently of CYP2C9 reduced-function alleles and its inclusion in the IWPC dosing algorithm accounting for 21% of warfarin dose variability. Although present in other ethnic groups, its impact on warfarin dose has been confirmed only in African Americans [22]. A novel haplotype CYP2C18 NM_000772.3: c.*31 T (rs2860840) and NM_000772.2: c.819 + 2182G 385 (rs11188059), referred to as "CYP2C:TG," encoding increased rate of metabolism of the CYP2C19 substrate, as escitalopram [23], sertraline [24], and omeprazole [25] was identified but not clopidogrel [26]. RNPGx classifies this SNP as Class 2 due to its influence on rate of metabolism of some CYP2C19 substrates.

3.1.6 | *CYP2C19*

Among the 35-star allele haplotypes defined by the Pharmacogene Variation Consortium (PharmVar) for the CYP2C19 gene, three have been ranked as Level 1 by the experts because of their allelic frequency in the general population and their established functionality: *CYP2C19*2* (**rs4244285**), *CYP2C19*3* (**rs4986893**), and *CYP2C19*17* (**rs12248560**). *CYP2C19*2* and *CYP2C19*3* alleles, both loss-of-function alleles, result in abnormal splicing for the first one and in a premature stop codon for the second one [27]. These predicted a poor metabolizer phenotype in homozygous *CYP2C19*2* or *CYP2C19*3* carriers' patients. Although the first one has a MAF ranging from 11% to 36% in the general population and up to 41% in the South Asian population, the second is mainly observed in the Asian population, with a MAF of up to 8% [28]. Moreover, a promoter variant (rs12248560) associated with an increased CYP2C19 gene expression defined the increased function *CYP2C19*17* allele, with a MAF of 1%–24% in the general population. These three variants are associated with Level 1A variant-drug annotations and CPIC guidelines for antidepressants (including selective serotonin reuptake inhibitors) [20], clopidogrel [29], mavacamten [30], voriconazole [31], and proton pump inhibitors [32].

The promoter variant (rs12248560) could lead to misinterpretation of the *CYP2C19*17* allele in rare cases because a second SNP suppressing the initiation codon (rs28399504) defines the nonfunctional *CYP2C19*4* allele [27]. After in vitro functional assessments [33], 12 other low or no function genetic variants of *CYP2C19*, characteristics of *CYP2C19*5*, **6*, **7*, **8*, **9*, **10*, **22*, **24*, **25*, **26*, and **35* (Table 2), are ranked as Class 2 variants because of low frequency in the general population. Finally, rare CYP2C19 structural variants are identified from cases of the Database of Genomic Variants and ClinGen, with ~1 in 2000 being a CYP2C19 full gene or partial-gene deletion carrier, designated by PharmVar as *CYP2C19*36* and **37* alleles, respectively [34]. Their detection could improve metabolizer phenotype prediction in the general population. The gene is thus eligible for Class 3 analysis.

3.1.7 | *CYP2C9*

Among the 85-star allele haplotypes defined by the Pharmacogene Variation Consortium (PharmVar) for the CYP2C9 gene, two variants are well known to experts because of their allelic frequency in the general population and their established functionality: *CYP2C9*2* (**rs1799853**) and *CYP2C9*3* (**rs1057910**). These two most common decreased function alleles among European patients impair the metabolism of warfarin by 30%–40% and 80%, respectively [35], reduce coumarin derivatives (warfarin and acenocoumarol) clearance, and are associated with a higher risk of bleeding [36]. Moreover, *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, and *CYP2C9*11* alleles, four of the most common CYP2C9 decreased function alleles among African populations contribute to warfarin dose variability [22]. The CPIC recommends genotyping *CYP2C9*2* and *CYP2C9*3* alleles in the European population and *CYP2C9*5*, *CYP2C9*6*, *CYP2C9*8*, and *CYP2C9*11* alleles in African populations to prevent bleeding risk and adapt the warfarin initial dose in CYP2C9 PM [9].

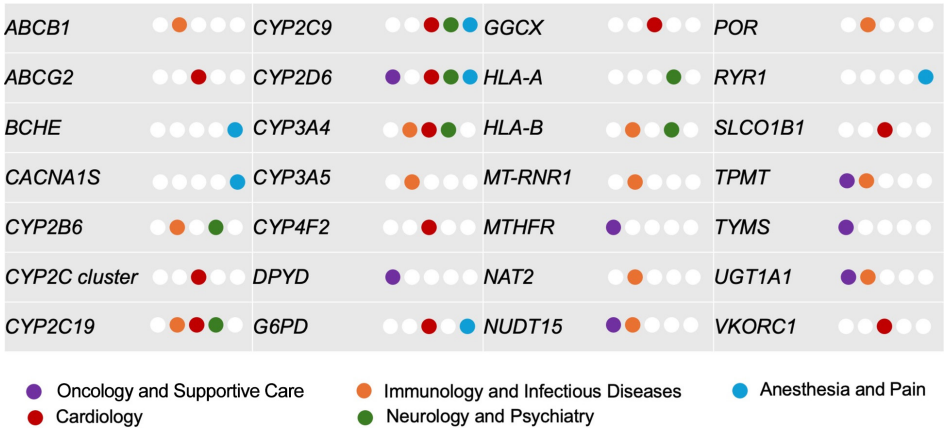

FIGURE 2 | Genes included in the RNPGx core panel and their recommended pharmacotherapeutic domains. Each colored dot represents a pharmacotherapeutic domain where the analysis of the corresponding gene is recommended.

The RNPGx classified *CYP2C9*2*, **3*, **5*, **6*, **8*, and **11* as Class 1 variants, whereas the other loss-of-function alleles **12, *13, *15, *24, *25, *33, *35, *39, *42, *43, *45*, and **52* may be investigated as a second-line approach (Class 2).

3.1.8 | *CYP2D6*

CYP2D6 is a key member of the cytochrome P450 superfamily involved in Phase I metabolism of several drugs [37]. CYP2D6 is mainly known to be involved in the metabolism of psychotropic (e.g., venlafaxine and paroxetine), analgesic (e.g., tramadol), antitumoral (e.g., tamoxifen), and cardiovascular (e.g., propafenone and metoprolol) agents. Moreover, *CYP2D6* is a highly polymorphic gene, with at least 164 star-alleles described to date (from **1* to **177*). In this context, the relationship between *CYP2D6* genotype and enzymatic activity, assessed through pharmacokinetic or clinical parameters, has been thoroughly documented [38, 39]. Thus, an activity score (AS) reflecting enzymatic activity (ranging from no function to increased function) can be assigned to alleles, enabling the identification of the phenotype group (from poor metabolizer to ultrarapid metabolizer) [40]. Consequently, allele selection was based on functional activity characterized by the AS and their population frequency. For Class 1, we retain alleles assigned to a modified phenotype (no function, decreased function, or increased function) and with a MAF greater than 0.10 in at least one ancestry group or 0.01 in the European population. Technical gene-specific limitations were also considered, leading to the exclusion of **36* structural variant from this category. Therefore, 12 single nucleotide polymorphisms (SNP) and copy number variation (CNV) research were selected: **rs16947**, **rs1135840**, **rs35742686**, **rs3892097**, **rs5030655**, **rs5030656**, **rs1065852**, **rs28371706**, **rs59421388**, **rs61736512**, **rs1058164**, **rs28371725**, and CNV (which enable the determination of *CYP2D6*2, *3, *4, *6, *9, *10, *17, *29, *41*, duplications/multiplications, and deletion, i.e., **5*).

For Class 2, the RNPGx recommends sequencing the coding exons of *CYP2D6* with the flanking regions (allowing the inclusion of splicing sites) along with hybrid genes. In order to align our recommendations with those proposed by a joint consensus of international organizations [41], particular attention should be given to the following SNP: rs5030867, rs5030865, rs5030862, rs774671100, rs72549352, rs267608319, rs72549356, rs72549346, rs1135822, rs72549347, rs79292917 (defining *CYP2D6*7, *8, *12, *14, *15, *21, *31, *40, *42, *49, *56*, and **59*), and structural variants (*CYP2D6*36*, *CYP2D6::CYP2D7*, and *CYP2D7::CYP2D6* hybrid genes). Nevertheless, the RNPGx recommends interpreting thoroughly all variants associated with modified phenotypes according to PharmVar [42]. When suitable high-throughput technologies are available, whole gene sequencing of the CYP2D locus, including promoter and UTR regions, corresponding to Class 3, would be more suitable. Of note, it is important to state that, due to the presence of different structural variants (such as CNV and hybrid genes) and closely related pseudogenes (*CYP2D7* and *CYP2D8*), *CYP2D6* sequencing is particularly challenging and requires great caution in its implementation [43].

3.1.9 | *CYP3A*

The human cytochrome P450 Family 3 Subfamily A (CYP3A), including CYP3A4 and CYP3A5, is located in a CYP3A gene locus on chromosome 7. CYP3A enzymes are important for metabolizing 30%–50% of clinical drugs in several domains, including immunoinfectiology, neuropsychiatry, and cardiology (Table 2). CYP3A4/CYP3A5 testing recommendations are recently synthesized by Pratt et al. [44], and it is considered that the CYP3A4 and CYP3A5 alleles recommended for inclusion in tier 1 include *CYP3A4*22* and *CYP3A5*3*, *CYP3A5*6*, and *CYP3A5*7*, and for inclusion in Tier 2, the *CYP3A4*20* allele.

For CYP3A4, the *CYP3A4*22* Intron 6 variant (**rs35599367**) has been associated with reduced enzyme activity and explained 12% of the variability in CYP3A4 enzyme activity among individuals [45]. The *CYP3A4*22* allele predominantly occurs in individuals of European ancestry with a MAF of 5% and American (MAF = 3%) and is less common among individuals of African (MAF < 0.1%) and Asian (MAF < 0.6%) ancestry. The loss of function *CYP3A4*20* allele is characterized by a single-nucleotide duplication within Exon 13 (**rs67666821**) that causes a frameshift and generation of a premature stop codon. The *CYP3A4*20* allelic frequency is high in the Hispanic population (3.8%) and is found among the Latin-American population at a frequency of 0.1%, whereas

TABLE 2 | Recommended Classes 1 and 2 regions of interest (ROIs) and gene eligibility for Class 3 analysis for RNPGx core selection. Identifiable Star Alleles or common haplotype name are given when applicable.

Core gene		Recommended Classes 1 and 2 ROIs	Eligibility for Class 3 analysis
ABCB1	Class 1	None recommended	Yes
	Class 2	None recommended	
ABCG2	Class 1	rs2231142	Yes
	Class 2	None recommended	
BCHE	Class 1	rs1799807 (A-variant), rs1803274 (K-variant)	Yes
	Class 2	None recommended	
CACNA1S	Class 1	rs1800559, rs772226819	No
	Class 2	None recommended	
CYP2B6	Class 1	rs3745274 (*9, *6 and several other alleles with reduced activity), rs28399499 (*18), rs2279343 (*4 or *6 with distinct functionality)	Yes
	Class 2	rs12721655 (*8, *13), rs36060847 (*12), rs34223104 (*22), rs34698757 (*28), rs34097093 (*28), rs281864907 (*38)	
CYP2C cluster	Class 1	None recommended	No
	Class 2	rs12777823, rs2860840, rs11188059	
CYP2C19	Class 1	rs4244285 (*2), rs4986893 (*3), rs12248560 (*17)	Yes
	Class 2	rs28399504 (*4), rs56337013 (*5), rs72552267 (*6), rs72558186 (*7), rs41291556 (*8), rs17884712 (*9), rs6413438 (*10), rs140278421 (*22), rs118203757 (*24), rs118203759 (*25), rs375781227 (*26), rs12769205 (*35)	
CYP2C9	Class 1	rs1799853 (*2), rs1057910 (*3), rs28371686 (*5), rs9332131 (*6), rs7900194 (*8), rs28371685 (*11)	Yes
	Class 2	rs9332239 (*12), rs72558187 (*13), rs72558190 (*15), rs749060448 (*24), rs1304490498 (*25), rs200183364 (*33), rs72558189 (*35), rs762239445 (*39), rs12414460 (*42), rs767576260 (*43), rs199523631 (*45), rs988617574 (*52)	
CYP2D6	Class 1	rs16947, rs1135840, rs35742686, rs3892097, rs5030655, rs5030656, rs1065852, rs28371706, rs59421388, rs61736512, rs1058164, rs28371725, and CNV (allowing identification of *2, *3, *4, *6, *9, *10, *17, *29, *41, *5 [deletion], and duplication/multiplication).	Yes
	Class 2	CDS; CYP2D7::CYP2D6 hybrid gene, CYP2D6::CYP2D7 hybrid gene	
CYP3A4	Class 1	rs67666821 (*20), rs35599367 (*22)	Yes
	Class 2	rs4986910 (*3), rs28371759 (*18)	
CYP3A5	Class 1	rs776746 (*3), rs10264272 (*6), rs41303343 (*7)	Yes
	Class 2	None recommended	
CYP4F2	Class 1	rs2108622 (*3 and *4)	No
	Class 2	None recommended	
DPYD	Class 1	rs3918290 (DPYD*2A), rs55886062, (DPYD*13), rs67376798 (c.2846A > T), rs115232898 (c.557A > G), rs75017182 (HapB3)	Yes
	Class 2	rs72549303 (*DPYD**3), rs72549309 (*DPYD**7), rs1801266 (*DPYD**8), rs1801268 (*DPYD**10), rs78060119 (*DPYD**12), rs1801160 (*DPYD**6), rs2297595 (c.496A > G), rs146356975 (c.868A > G), rs112766203 (c.2279C > T), rs186169810 (c.1314 T > G), rs72549304 (c.1475C > T), rs59086055 (c.1774C > T), rs55674432 (c.2639G > T)	

(Continues)

TABLE 2 | (Continued)

Core gene		Recommended Classes 1 and 2 ROIs	Eligibility for Class 3 analysis
G6PD	Class 1	None recommended	Yes
	Class 2	None recommended	
GGCX	Class 1	None recommended	No
	Class 2	rs11676382	
HLA-A	Class 1	HLA-A*31:01, HLA-A*02:01	No
	Class 2	None recommended	
HLA-B	Class 1	HLA-B*15:02, HLA-B*15:11, HLA-B*58:01, HLA-B*57:01	No
	Class 2	HLA-B*13:01	
MT-RNR1	Class 1	rs267606619, rs267606618, rs267606617	No
	Class 2	None recommended	
MTHFR	Class 1	None recommended	No
	Class 2	rs1801133, rs1801131	
NAT2	Class 1	rs1208, rs1801280, rs1799930, rs1799931, rs1801279, rs4986996, rs72554616, rs1805158, rs1799929, rs1041983, rs56054745 (*4 *16 *12, *5 *16, *6, *7, *14, *12D, *17, *19, *11 *12A,B,C, *12A,B,C *13, *18)	Yes
	Class 2	None recommended	
NUDT15	Class 1	rs746071566, rs116855232 (*2 *6 *9, *2 *3)	Yes
	Class 2	None recommended	
POR	Class 1	None recommended	No
	Class 2	rs1057868 (*28)	
RYR1	Class 1	None recommended	Yes
	Class 2	None recommended	
SLCO1B1	Class 1	rs4149056 (*5, *15, *46, *47), rs2306283 (*14, *15, *31, *37, *46, *47), rs11045819 (*14), rs59502379 (*9, *31)	Yes (*48, *49)
	Class 2	rs373327528 (*23)	
TPMT	Class 1	rs1800462, rs1800460, rs1142345, rs1800584 (*2, *3B, *3C, *4)	Yes
	Class 2	rs72552738 (*11), rs9333569 (*14), rs9333570 (*15), rs74423290 (*23), rs267607275 (*29), rs1142345 (*41)	
TYMS	Class 1	None recommended	No
	Class 2	rs45445694 (2RC), rs2853542 (3RC), rs11280056	
UGT1A1	Class 1	rs4148323 (*6), rs3064744 (*28 *36 *37)	Yes
	Class 2	None recommended	
VKORC1	Class 1	rs9923231, rs9934438	Yes
	Class 2	None recommended	

the frequency is <0.03% in European (non-Finnish) and African/African American populations.

The data for the association of CYP3A4 genetic variants with drug response are most consistent for quetiapine, an atypical antipsychotic indicated for the treatment of schizophrenia and bipolar disorder [46].

For CYP3A5, the *CYP3A5*3* no function allele, defined by the intronic variant (**rs776746**), is associated with poor metabolism (historically also known as the nonexpressor phenotype). It has a MAF of 94% in European individuals and 18% in African individuals. MAF ranges between 67% and 71% in South and East Asian, respectively. Two other no function alleles, *CYP3A5*6* (**rs10264272**) and *CYP3A5*7* (**rs41303343**), occur predominately

in individuals of the African population, with reported MAFs of 5%–24% and 9%–14%, respectively.

Practice guideline for CYP3A5 genotype-based dosing for tacrolimus is provided by the CPIC [47] and recommendations from RNPGx [48].

RNPGx classifies these SNPs as Class 1 due to its PharmGKB Level 1A annotation for CYP3A4/quetiapine and CYP3A5/tacrolimus. Two other *CYP3A4* alleles (**3* and**18*) are classified as Class 2 due to their PharmGKB Level 2 tacrolimus. The existence of reported rare variants justifies considering a Class 3 approach for this gene.

3.1.10 | *CYP4F2*

Vitamine K is metabolized by hepatic CYP4F2 enzyme to hydroxy-vitamine K1 and removed from the vitamin K cycle. This enzyme limits vitamin K excessive accumulation. The SNP *CYP4F2*3* (c.1297G > A, p.V433M, **rs2108622**), found in *CYP4F2*3* and **4* alleles, decreases enzyme CYP4F2 activity [49] and increases variability in warfarin dose in European and Asian populations [50]. RNPGx classifies this SNP as Class 1 due to its PharmGKB Level 1A annotation. Meta-analysis shows that the *CYP4F2*3* allele is associated with interindividual variability in response to coumarin drugs, but with a lower effect size (8%–11% higher warfarin doses in allele A carriers) than those contributed by VKORC1 and CYP2C9 polymorphisms [51]. Another nonsynonymous genetic variant (rs3093015) included is in *CYP4F2*2* and *4 alleles, found frequently in both African and European American sampling panels (23%–40% MAF), without any functional effect on the ω-hydroxylation of arachidonic acid or leukotrienes LTB_4 [49]. This variant has not been retained in the RNPGx selection.

3.1.11 | *DPYD*

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme involved in nearly 85% of the catabolism of fluoropyrimidines antimetabolite drugs (FP), including 5-fluorouracil (5-FU), capecitabine, and tegafur, which are widely used to treat solid tumors (colorectal, breast, pancreatic, head, and neck). DPD protein is encoded by the *DPYD* gene, which is located on chromosome 1p21.3, spans 843 317 bp in length, and contains 23 exons. Over 1598 sequence variants in the *DPYD* gene have been identified according to Genome Aggregation Database version 4.0.0, but the functional and clinical roles remain uncertain for most of these variants, especially those that are rare (DPYD | gnomAD v4.1.0 | gnomAD).

It has been thoroughly demonstrated that preemptive *DPYD* genotyping avoids potentially life-threatening toxicity in patients carrying *DPYD* deleterious germline variants. Hence, the CPIC has developed guidelines based on a *DPYD* activity score to determine the predicted DPD phenotype. Briefly, normal function variants have an activity value of 1, whereas a value of 0 defines nonfunctional variants, and decreased activity variants are assigned a value of 0.5.

In the same way, to identify patients at an elevated risk of FP toxicity, the RNPGx considers as a first tier (1) uracilemia as a more sensitive and accurate biomarker of DPD activity and recommends in conjunction (2) targeted *DPYD* genotyping when biochemical phenotyping is not contributing (e.g., uracilemia close to the cutoff value, discordance between uracilemia and dihydrouracil/uracil ratio, and renal dysfunction). Taking into account the recent joint AMP, CPIC, and DPWG recommendations [52], AOIM guidelines [53], two limited sets of *DPYD* variants to test as Class 1 or 2 were retained (Table 2). The core panel consists of five variants with well-established functional impact and significant allele frequencies in various populations, including c.1905 + 1G > A (*DPYD**2A, **rs3918290**—MAF: 0.3% in the whole population; 0.1% in African; 0.5% in European; and 0.8% in South Asian populations), c.1679 T > G (*DPYD**13, p.I560S, **rs55886062**—MAF: 0.02% in the whole population and 0.1% in the European non-Finnish population), c.2846A > T (**rs67376798**, p.D949V—MAF: 0.2% in the whole population; 2.3% in African; 2% in Utah residents with European ancestry; and 0.3% in other European populations), c.557A > G (**rs115232898**, p.Y186C—MAF: 0.6% in the whole population; 2.3% in African; 0% in European; and 0.3% in American populations), and c.1129-5923C > G (**rs75017182**, *HapB3*—MAF: 1% in the whole population; 0.1% in African; 2.4% in European; 0.6% in American; and 1.9% in South Asian populations). Additionally, the secondary panel includes other variants with lower allele frequencies or uncertain but potentially deleterious effects (Table 2).

Given the contribution of rare deleterious variants to FP toxicity published in many case reports, such as c.2872A > G recently described by de Haar-Holleman et al. [54], sequencing of all coding regions (Class 3) is encouraged for the *DPYD* gene.

Regarding the *DPYD* copy number variation, RNPGx does not currently have recommendations for routine clinical testing. However, deletions in Exons 4, 6, 9–10, 12, and 14–16, predicted to result in complete loss of function, have been identified [52, 55, 56], which emphasizes the importance of *DPYD* coding regions sequencing over targeted genotyping to detect both common and rare variants, as well as structural variants. In case multiple variants are detected, RNPGx underscores the necessity of determining their phase to accurately assess their combined functional impact [54].

3.1.12 | *G6PD*

The G6PD gene encodes glucose-6-phosphate dehydrogenase, a key enzyme in the pentose phosphate pathway that protects red blood cells from oxidative stress by producing NADPH. G6PD deficiency, caused by genetic variants, predisposes individuals to episodes of acute hemolytic anemia (AHA) triggered by oxidative stress from certain drugs (Table 2), infections, or foods (notably fava beans). This X-linked disorder primarily affects males, with a global prevalence of approximately 5%, rising to 30% in malaria-endemic regions because the heterozygous state (females) confers protection from severe infection by *Plasmodium falciparum* and possibly *Plasmodium vivax* [57]. In Europe, the World Health Organization (WHO)

estimated the prevalence at 0.4%, but this figure is presumably underestimated due to population migrations from high-prevalence regions and the increasing genetic admixture observed in contemporary European populations. Over 230 G6PD variants have been identified, most of which are SNPs [58]. Historically, the WHO classified these variants into four phenotypic categories (I–IV).

However, in 2022, a new classification was proposed, dividing variants into four classes: A (<20% activity with chronic hemolysis), B (<45% activity with acute, triggered hemolysis), C (60%–150% activity with no hemolysis), and U (uncertain clinical significance). The CPIC provides recommendations for drug use in the context of G6PD genotype, including stratification of drug risk based on enzymatic activity [59]. Given the number of variants associated with deficiency, sequencing methods (Class 3) are suggested as the first-line approach for comprehensive and accurate detection by RNPGx.

3.1.13 | *GGCX*

Gamma-glutamyl carboxylase (GGCX) catalyzes the posttranslational carboxylation of vitamin K-dependent blood coagulation factors to make them functionally active, with the reduced vitamin K serving as an essential cofactor. Some rare nonsynonymous GGCX variants are associated with clotting disorders; thus, some GGCX genetic variants were investigated for their influence on warfarin dose variability [60]. One variant, rs11676382, is associated with a 6% reduced warfarin dose per G allele [10] and is assigned as Class 2 by RNPGx.

3.1.14 | *HLA*

Despite the great polymorphism of the HLA system, only a limited number of alleles have been described in the literature as having a link with the onset of adverse drug reactions through the triggering of immune responses. As the frequency of HLA alleles differs from one population to another, it is interesting to confirm these links in studies of populations of different origins.

A case in point is B*57:01/abacavir, first published in 2002 and confirmed in 2008 by other teams. This allele is most common in the Caucasian (MAF around 6%) and African-American (2%–3%) populations.

Among people with abacavir hypersensitivity, 44% of Caucasians and 100% of African-Americans were positive for the HLA-B*57:01 allele [61]. The European Medicines Agency, the US Food and Drug Administration, and the FDA recommend testing for HLA-B*57:01 before starting treatment. In this context, PharmGKB and RNPGx classify it as Level 1A and Class 1, respectively.

At the same level of recommendation, authorities recommend testing the presence of A*31:01, HLA-B*15:02, and B*15:11 before starting carbamazepine, oxcarbazepine, phenytoin, and lamotrigine in Asian populations where these alleles are more frequent, in view of the increased risk of Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [62].

Similarly, initially identified as a genetic marker for allopurinol-induced SCAR in a Taiwanese study [63] (incidence 0.4% of new users, half hospitalized, and high mortality rate [0.39/1000]), the association with B*58:01 was subsequently validated in numerous ethnic populations worldwide (Thailand, Japan, South Korea, Hong Kong, Australia, Portugal, and Europe). The American College of Rheumatology has therefore been recommending HLA-B*58:01 genetic screening for new allopurinol users in Asian populations since 2012.

The strong association of the HLA-B*13:01 allele with dapsone-induced SCAR has been well established in the Asian population, particularly in the Chinese and Southeast Asian populations, but has not been identified in the European, Caucasian, American, African, or Oceanic populations, either because of a lower frequency of the allele or perhaps because it is less prescribed and, therefore, fewer studies or cases are reported in the literature. This is why PharmGKB and RNPGx are aligned on a Level 2a/Class 2 [64]. Conversely, it is interesting to note that certain targeted therapies in oncology have been designed to be adapted to a population carrying the most frequent allelic sequence (A*02, e.g., Tedopi, Phase 3 study) or even a specific allele (A*02:01, e.g., Tebentafusp, marketing authorization).

The search for these alleles makes them a prerequisite for defining a patient's eligibility for treatment and therefore a companion diagnosis. This is why the RNPGx is proposing to test A*02:01 in oncology, Class 1, for existing and future therapies.

3.1.15 | *MT-RNR1*

MT-RNR1 is a mitochondrial gene encoding the small subunit of the mitochondrial ribosome (12S rRNA). Specific variants in this gene predispose individuals to highly prevalent ototoxicity following aminoglycoside administration, regardless of the specific aminoglycoside used.

Three Class 1 variants were prioritized by RNPGx for this gene: **rs267606617** (m.1555A>G), **rs267606619** (m.1494C>T), and **rs267606618** (m.1095T>C). These variants exhibit demonstrated functional impacts by enhancing the aminoglycosides' affinity for 12S rRNA or by enhancing apoptosis after administration. The population frequencies range from 1/1000 to 1/10000. Additionally, numerous pedigrees with maternal inheritance of these variants have been documented in the literature [65]. PharmGKB has classified these three variants as Level 1A, and RNPGx emphasizes the importance of testing for them when aminoglycoside administration is anticipated or in the context of posttoxicity investigations [65]. RNPGx recommendations focus exclusively on these three MT-RNR1 variants for clinical testing.

3.1.16 | *MTHFR*

The methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to

5-methyltetrahydrofolate (a cosubstrate for homocysteine remethylation to methionine), which can in turn be converted to tetrahydrofolate. The human *MTHFR* gene is located on chromosome 1p36.22 and is composed of 12 exons.

Frosst et al. [66] identified a c.677C > T mutation in the *MTHFR* gene, resulting in a p.Ala222Val substitution, but this polymorphism is actually referred to as c.665C > T (rs1801133). The allele frequency of the substitution is about 24.5% in the whole population, 36% in European, 47% in American, 9% in African, and 30% in East-Asian individuals. Patients homozygous for this mutation undergo a decreased activity of 70% and present significantly elevated plasma homocysteine levels. The rs1801133 variant may thus represent an important genetic risk factor in vascular disease. A second polymorphism c.1286A > C (legacy name c.1298A > C, rs1801131—MAF: 25% in the whole population; 15% in African and American; 22% in East-Asian; 31% in European; and 42% in South-Asian individuals) is less relevant, as it reduces the activity of MTHFR by only 40% in homozygous individuals.

The toxicity of methotrexate might be linked to a decrease in intracellular tetrahydrofolate concentrations. Hence, as methotrexate inhibits dihydrofolate reductase, which converts dihydrofolate to tetrahydrofolate, the presence of rs1801133 could increase the toxicity risk. Subsequently, the need for folic acid could be increased. Although the DPWG did not conclude that *MTHFR* variants are a clinical interest for methotrexate therapy adjustment [67], the Canadian HCSC indicates in the product monograph of norelgestromin and ethinyl estradiol (EVRA) that the drugs are contraindicated in women with the presence of severe or multiple risk factor(s) for arterial or venous thrombosis such as hyperhomocysteinemia (e.g., due to rs1801133 in the *MTHFR* gene). RNPGx thus classified rs1801133 and rs1801131 as Class 2 variants.

3.1.17 | *NAT2*

The *NAT2* gene (1 coding exon—873 bps) encodes the enzyme N-acetyltransferase type 2 (290 aminoacids), which plays a critical role in the metabolism of various xenobiotics.

Genotyping the *NAT2* gene is of interest for improving therapeutic efficacy while minimizing adverse effects, especially for isoniazid treatment. Genetic polymorphisms in *NAT2* significantly influence the rate of acetylation, categorizing individuals as rapid, intermediate, or slow acetylators. This classification is based on the genotyping of 11 common SNPs: **rs1208**, **rs1801280**, **rs1799930**, **rs1799931**, **rs1801279**, **rs4986996**, **rs72554616**, **rs1805158**, **rs1799929**, **rs1041983**, and **rs56054745**. RNPGx classifies these SNPs as Class 1 due to its PharmGKB Level 1B annotation for NAT2/isoniazid. For instance, isoniazid, commonly used to treat tuberculosis, can cause peripheral neuropathies and hepatotoxicity in slow acetylators (alleles *NAT*5-*6-*7*, for example). Genotyping *NAT2* allows the identification of patients at risk of isoniazid-induced peripheral neuropathy and hepatotoxicity (particularly slow acetylator carriers of *NAT2* *5, *6, or *7 alleles), enabling dose adjustments or the selection of safer alternative therapies. Practice guidelines for *NAT2* genotype-based dosing before isoniazid treatment are supported by several studies that show a significantly reduced incidence of hepatotoxicity [11].

3.1.18 | *POR*

The POR gene, short for P450 (cytochrome) oxidoreductase, encodes an enzyme called cytochrome P450 oxidoreductase, which is essential for the proper function of CYPs enzymes. Its primary role is to transfer electrons from NADPH (nicotinamide adenine dinucleotide phosphate) to all microsomal P450 enzymes, enabling them to catalyze various chemical reactions including the biotransformation of xenobiotics. Rare mutations in the POR gene can lead to a spectrum of health issues. One notable condition is congenital adrenal hyperplasia, which affects steroid hormone production and can lead to problems with sexual development and adrenal gland function. Additionally, POR gene variants may also be involved in PGx [68]. The main PGx variant studied so far is c.1508C > T, p.A503V, rs1057868, and *POR*28*.

Studies have shown that the impact of this variant depends not only on the nature of the CYP isoforms but also on the nature of the substrate considered. Indeed, this variant was shown to significantly reduce CYP3A5 activity, but not CYP3A4 activity, towards midazolam [69]. Conversely, it significantly increased CYP3A5 activity towards tacrolimus, again without affecting CYP3A4 activity towards the same substrate [12].

3.1.19 | *RYR1* and *CACNA1S*

Variants in *CACNA1S* and in RYR1 genes have been found to be associated with Malignant Hyperthermia Susceptibility (MHS) related to inhaled anesthetics (sevoflurane, halothane, enflurane, isoflurane, methoxyflurane, and desflurane) or succinylcholine. In addition, there is some evidence that the risk of rhabdomyolysis in individuals taking statins for hypercholesterolemia increased with some RYR1 and CACNA1S variants [70].

The *CACNA1S* gene lies on Chromosome 1, spans 73 kb, is composed of 44 exons, and encodes the α 1 s subunit of the dihydropyridine receptor (DHPR), which functions by activating RYR1 channels. The 161.6 kb RYR1 gene is located on chromosome 19, includes 106 exons, and encodes the ryanodine receptor isoform 1 protein (RYR1).

In any individuals known to harbor pathogenic RYR1 or CACNA1S variants, the risk for a Malignant Hyperthermia event can be avoided by using nontriggering anesthetics [71].

Among the 8787 RYR1 variants known to date, the EMHG consortium set a list of 66 variants considered as "diagnostic mutations" (https://www.emhg.org/recommendations-1/2025/1/6/use-of-penthrox-methoxyflurane) and 2 very rare mutations of interest (with MAF < 0.01%) in the *CACNA1S* gene (**rs1800559** and **rs772226819**).

The ClinGen RYR1 Variant Curation Expert Panel from the ACMG and AMP has classified a total of 335 variants, including 29 considered pathogenic, 57 likely pathogenic, 17 likely benign, 13 benign, and 219 of uncertain significance variants [72]. Both initiatives should ease the interpretation burden for reporting diagnostic mutations. The true predicted prevalence of MH-associated RYR1 pathogenic variants should lie between 1 in 300 and 1 in 1075 individuals. The RNPGx is confident in the work of both consortia and recommends following their guidelines. RNPGx retained two Class 1 variants in *CACNA1S* (**rs1800559** and **rs772226819**). Given the number of variants to consider in *RYR1*, a Class 3 approach is recommended for this gene.

3.1.20 | *SLCO1B1*

Among the 44 alleles described on the Pharmacogene Variation Consortium (PharmVar), only 5 *SLCO1B1* (solute carrier organic anion transporter family member 1B1) alleles have to be investigated in the first intention, due to their clinically relevant functional impact, and were ranked as Class 1 for RNPGx: *SLCO1B1*14* allele classified as increased function, *SLCO1B1*1* and *SLCO1B1*37* alleles classified as normal function and loss-of-function *SLCO1B1* 5*, *9*, and *15* alleles. The *SLCO1B1* 5*, *9*, and *15* alleles are associated with decreased OATP1B1 hepatic transporter function impairing statin hepatic uptake [73], increasing the systemic exposure to statin [74] and the risk of statin-associated musculoskeletal symptoms (SAMS) [75]. The most common nonsynonymous c.521 T > C variant, **rs4149056**, identified by GWAS and associated with the occurrence of SAMS, is contained within *SLCO1B1*5* and *15* alleles, with MAF around 14%–21% in European and American populations. Although the nonsynonymous genetic variant c.388A > G (**rs2306283**) is common to the *SLCO1B1*14*, *15, and *37* alleles, the identification of the genetic variant c.463 C > A (**rs11045819**), also constituting the *SLCO1B1*14* haplotype, enables it to be discriminated from the normal function *SLCO1B1*37* allele.

The nonsynonymous c.1463G > C (**rs59502379**) defines the *SLCO1B1*9* allele, more frequent in the African American population (MAF = 4%). This rs59502379 is also present, along with rs2306283, in the *31, *46, and *47 loss-of-function alleles. *SLCO1B1*23* is another loss-of-function allele characterized by an isolated SNP occurring at low frequency in European populations. It is ranked as Class 2 by RNPGx because it could be genotyped to explain SAMS in second intention. Finally, some rare low or no function genetic variants of SLCO1B1, which were associated with reduced methotrexate clearance, another OATP1B1-transported drug, could be genotyped [76]. Rare *SLCO1B1* structural variants are identified from cases of the Database of Genomic Variants, with a frequency of about 0.07% and 0.06% being *SLCO1B1* partial-gene or full gene deletion, respectively, designated by PharmVar as *SLCO1B1*49* and *48 alleles, respectively. Complete OATP1B1 deficiency due to a 405-kb deletion gene causes human Rotor syndrome, leading to the interruption of conjugated bilirubin reuptake into the liver [77] and should be researched with large sequencing technology (i.e., Class 3).

3.1.21 | *TPMT* and *NUDT15*

Thiopurine-S-methyltransferase (TPMT), located on chromosome 6p22.3 (NM_000367.5), and Nudix hydrolase 15 (NUDT15), located on chromosome 13q14.2, encode two key enzymes involved in the metabolic pathway of thiopurine medications (azathioprine, 6-mercaptopurine, and 6-thioguanine) commonly prescribed as chemotherapeutic agents for the treatment of acute lymphoblastic leukemia, as immunosuppressant agents for the treatment of autoimmune disorders, as well as to prevent organ transplant rejection. Genetic variations in these two genes would explain approximately 45% of the interpatient variability in thiopurine-induced myelosuppression. TPMT variants are found predominantly in White patients, whereas NUDT15 variants are prevalent in individuals of Hispanic or Asian origin.

Among the 46-star allele haplotypes currently referenced for TPMT [78], 11 are associated with a loss of function. RNPGx classified five alleles (corresponding to five individual variants) as Class 1 (Table 2). *TPMT*2* (**rs1800462**), *3A (**rs1800460** and **rs1142345**), *3B* (**rs1800460**), and *3C* (**rs1142345**) alleles were found in the Latino/European populations at MAF of 0.35%/0.2%, 4.2%/3.4%, 0.2%/0.3%, and less than 1%, respectively. *TPMT*2* is found more commonly in individuals of African ancestry (MAF = 0.5%), whereas the *TPMT*3C* allele is most common in the African and East Asian populations (MAF = 7% and 2%, respectively). The *TPMT*4* allele (**rs1800584**) is a rare allele, with a frequency of 0.01% in the European population. The other six alleles are considered Class 2 (*TPMT*11*, *14, *15, *23, *29, and *41*).

Regarding NUDT15, two SNPs are considered of major interest: **rs116855232** and **rs746071566** and classified as Class 1 by RNPGx. The **rs116855232** variant is a missense variant located in Exon 3 of the *NUDT15* gene (c.415C > T, p.Arg139Cys). It is found in the nonfunctional *NUDT15*3* allele, which is common in South Asian (MAF of 7%) and East Asian (10%) populations but has a frequency below 1% in Latin American and European populations.

The **rs746071566** variant corresponds to a six-base pair insertion (GAGTCG) in Exon 1 of the *NUDT15* gene (c.50_55dup, p.Gly17_Val18dup). The *NUDT15*6 allele* is characterized by the presence of four copies of this repeat sequence. It has a frequency of approximately 5% in the East Asian population but remains below 0.5% in European, Central/South Asian, and Latin American populations. The Association for Molecular Pathology (AMP) has classified it as Tier 2, despite its uncertain functional significance, as its detection is essential for accurately distinguishing *NUDT15*2* from *NUDT15*3 (NUDT15*2* contains both **rs746071566** and **rs116855232** whereas *NUDT15*3* contains only **rs116855232**). If only the second is analyzed, it is recommended that the genotype be reported as "*NUDT15*2* or *NUDT15*3*" [79].

The *NUDT15*2* allele is found at frequencies of 3.7% in Latin American populations and 3.5% in East Asian populations. The GAGTCG repeat sequence of **rs746071566** is also present in two copies in the *NUDT15*9* allele, which has a frequency of 0.2% in the European population and below 0.05% in Central/South Asian and Latin American populations.

3.1.22 | *TYMS*

The major target of the 5FU is thymidylate synthase (TS). TS is encoded by the *TYMS* gene, located on chromosome 18p11.32, consisting of seven exons and spanning 16 kb in length. A 28-bp variable number of tandem repeats (rs45445694—MAF: 0.08% in the whole population; 2.6% in African/African American; 0.04% in European non-Finnish; and 0.1% in East-Asian populations) in the nontypical 5′UTR promoter region (TYMS enhancer region or TSER) has been reported to affect transcription [80]. Most patients have either two (2R) or three repeats (3R) in this regulating region. As many as nine TSER repeats have been reported in some rare cases [81]. The so-called "Upstream Stimulatory Factor 1" (USF1) transcription factor has been shown to bind to the consensus recognition domains in the TSER to activate TYMS transcription. The number of USF1-binding sites varies depending on the number of repeat sequences and determines the gastrointestinal toxicity risk [82]. It was classically described that at Position 12 of the second repeat of the 3R allele, a G > C SNP (rs2853542—MAF: 44% in the whole population; 26% in African; 50% in European non-Finnish; 50% in American; and 43% in East-Asian populations) reduces transcription by abolishing an (USF1) binding site. Although the SwissMedic fluorouracil drug label indicates that in patients 2R/2R the probability of Grade 3–4 toxicity is 20 times higher and in patients 2R/3R six times higher than in patients 3R/3R (Annotation of Swissmedic Label for fluorouracil and TYMS), this association is still controversial. RNPGx thus classified rs45445694 and rs2853542 as Class 2 variants.

In addition, a 6-bp deletion in the 3′UTR (rs11280056) has been extensively studied in patients with methotrexate or FP regimen, but the results are still conflicting [83]. The RNPGx considers this polymorphism as potentially useful and classifies it as a Class 2 variant.

3.1.23 | *UGT1A1*

High unconjugated bilirubin level known as Gilbert's syndrome is a consequence of Uridine diphosphate glucuronosyl transferase 1A1 (UGT1A1) deficiency. UGT1A1 is mainly expressed in the liver and the gut. It is encoded by the UGT1A complex locus. The *UGT1A1* gene is located on Chromosome 2 (2q37.1) and spans 13 kb in length. It consists of five exons, of which the first exon at the 5′ terminus is unique and Exons 2–5 are common to nine *UGT1A* transcripts with unique 5′ ends and identical 3′ ends. Among the 943 alleles described to date for *UGT1A1* (gnomADv4.1.0), a "TA" repeat in the promoter region is of particular interest. Indeed, a 67%–82% decrease in gene expression is linked to seven "TA" repeats instead of six (referred to **28* in the **rs3064744** variant). When the "TA" number is 5, the expression level of UGT1A1 is increased, resulting in enhanced enzymatic activity (referred to **36* in the **rs3064744** variant). A second relevant missense mutation within Exon 1 (c.211G > A; p.Gly71Arg) decreases the enzymatic activity (referred to **6* or **rs4148323**). The frequency of the various UGT1A1 alleles shows considerable interethnic variability. The prevalence ranges from 12% (European non-Finnish) to 35% (South Asian populations) for **28* and is about 14% in East-Asian populations for **6* (0% in African, 1% in American, and European individuals).

The most efficient way to eliminate the SN-38 (irinotecan's active metabolite) is glucuronidation by UGT1A1. The risk of severe irinotecan toxicities, such as diarrhea or febrile neutropenia, is linked to an increase in SN-38 concentrations due to UGT1A1 deficiency.

The RNPGx/GPCO guideline recommends (1) genotyping before starting irinotecan (2) to adjust the dose with respect to regimen and genotype [84]. Based on a genotype–phenotype translation system and a "Clinical Implication Score" for UGT1A1/Irinotecan, the DPWG considers (1) to genotype all patients treated with irinotecan, even at low doses, and (2) to start with a 30% dose decrease in poor metabolizers [85]. Decreased function genotypes of UGT1A1 may affect toxicity and/or tolerability of other drugs. Such drugs include the antiprotease atazanavir, oncology medications (belinostat and sacituzumab govitecan), or antimalaria drugs such as pegvisomant. For example, in a poor metabolizer with decreased UGT1A1 activity, there is a high likelihood of developing jaundice that results in atazanavir discontinuation [86]. RNPGx classified two variants as Class 1 and recommends a Class 3 approach for further analysis (Table 2).

3.1.24 | *VKORC1*

The target enzyme of coumarin derivatives is the vitamin K epoxyde reductase protein (coding by VKORC1), which regulates the step limiting in vitamin K recycling. A common VKORC1 c.-1639G > A variant, **rs9923231**, located in the promoter region of the VKORC1 gene, affects VKOR protein expression and is associated with greater sensitivity to warfarin in Asian, African, and European populations [87]. VKORC1 c.-1639G > A variant is on linkage disequilibrium between five other SNPs as demonstrated by Rieder; the correspondence values for c.-1639G > A were calculated in Caucasian ($R^2 = 0.967$), Asian ($R^2 = 0.996$), and African American ($R^2 = 0.815$) populations with VKORC1 c. 1173 C > T (**rs9934438**) located in intron 1 *VKORC1* [88]. Genotyping VKORC1 c.1173 C > T could be useful to explain bleeding events in some African American patients treated with low warfarin doses and c.-1639GG VKORC1 carriers. RNPGx classifies these two SNPs (rs9923231 and rs9934438) as Class 1, due to its PharmGKB Level 1A annotation and in accordance with AMP PGx working group, to prevent bleeding risk and adapt warfarin dose [89].

Multiple rare nonsynonymous SNPs (including rs104894539, rs61742245, rs104894540, rs104894541, rs72547529, rs72547528, and rs104894542) in *VKORC1* confer warfarin resistance and must be identified preferably through sequencing techniques (Class 3 approach) in case of high warfarin dose intake associated with low INR values [90].

4 | Discussion

From an initial list of 81 genes, a group of 19 experts from the RNPGx network evaluated gene relevance across five therapeutic areas using a structured, evidence-based approach. Their work was independently reviewed by three additional RNPGx members and further adjusted by seven external experts

consulted on specific points. This process led to the identification of a core selection of 28 genes that should be included in any pharmacogenetic panel intended for clinical use.

Specific regions of interest (ROIs) were defined for each of the 28 selected genes of the core panel. ROIs were categorized into three analytical classes according to clinical utility and compatibility with available technologies.

In total, 76 and 62 variants (plus CYP2D6 CDS and hybrid genes analysis) were retained in Classes 1 and 2, respectively, across the 28 core genes. Class 1 variants are SNP for the vast majority but also include six HLA variants and CNV events for CYP2D6 (i.e., gene deletion and duplication/multiplication). Class 2 contains one HLA allele.

Notably, *CYP2D6* represents a particular case within Class 2. Due to the complexity of its locus, including numerous star alleles defined by SNPs, CNVs (e.g., deletion and duplication), and hybrid genes, standard genotyping is often insufficient. Full sequencing of the coding region with flanking introns is already recommended to ensure accurate characterization.

Given its high polymorphism and the presence of closely related pseudogenes, whole-locus sequencing (Class 3) may be preferable when technically feasible.

Class 3 eligibility was retained for 18 genes. This means that RNPGx considers extended sequencing to be relevant for these 18 genes out of the 28 in the core panel. For the remaining genes, the added value of such analysis was considered more limited. In many cases, particularly for cytochrome P450 genes, Class 3 selection was motivated by the need to accurately identify star alleles. For genes such as *DPYD*, the inclusion of Class 3 reflects the contribution of rare variants with functional impact. Gene size was also taken into account when evaluating feasibility and expected yield. In some specific cases, such as *SLCO1B1*, the recommendation is supported by the presence of copy number variation. This three-level classification allows flexible adaptation to diverse laboratory workflows and supports implementation through various technological platforms, from targeted genotyping to high-throughput sequencing.

Compared to existing panels used in recent studies, the RNPGx core selection offers broader coverage in both genes and variants. For instance, the pharmacogenetic panel used in the PREPARE study included 12 genes and covered 50 variants, whereas the INGENIOUS trial relied on a 13-gene panel analyzing 43 variants [2, 3]. These panels were designed as fixed-content assays, based on the clinical evidence and genotyping technologies available at the time. In contrast, the RNPGx recommendations propose a tiered and flexible structure, in which each gene has Class 1, 2, or 3 according to the current levels of evidence and clinical applicability.

This approach is intended primarily for clinical use in situations where pharmacogenetic testing is already indicated and does not imply systematic preemptive testing of all listed genes or variants. Class 1 variants correspond to those that may be used in routine care, including preemptively in well-defined clinical contexts. Class 2 variants are optional and may be considered when Class 1 testing does not explain a patient's response or in exploratory or research settings. Class 3 reflects an analytical strategy based on broader sequencing or structural variant analysis, whose use depends on laboratory capabilities and clinical timelines rather than systematic implementation. This gradation allows for flexible implementation, ranging from minimal, high-priority variant sets to more comprehensive or exploratory analyses depending on clinical context or technological capacity. Such a framework will be useful for targeted clinical applications and broader use cases, including the reanalysis of exome or genome data. Importantly, the cost-effectiveness of testing was not assessed in this work, as it is highly dependent on local organization and testing strategy; in particular, costs may be negligible when pharmacogenetic information is secondarily extracted from existing WES/WGS data generated for diagnostic purposes. Overall, the proposed structure is consistent with the evolution of knowledge and the growing feasibility of integrating PGx into routine care but can also be adapted according to economic and organizational constraints when selecting the level of analysis.

Some limitations should be acknowledged. The selection process focused on a defined set of clinical scenarios, which may have excluded certain therapeutic areas where pharmacogenetics is relevant but less frequently encountered in routine care. For example, eliglustat, used in Gaucher Disease Type 1 (a genetic disorder), was not considered in this work, although CYP2D6 genotyping is mandatory for its prescription. Similarly, lonafarnib, a drug indicated for Hutchinson–Gilford progeria syndrome, includes CYP3A-based dosing guidance in the FDA label. Although these two drugs were not explicitly considered during the gene selection process due to their restricted and highly specialized use, their associated genes (i.e., CYP2D6 and CYP3A) are included in the RNPGx core panel, and the recommendations for these genes apply to these drugs. This illustrates that the RNPGx framework might be helpful, even for additional drugs or rare indications not explicitly considered during panel design.

Another limitation concerns the clinical applicability of Class 2 variants. Their clinical relevance may require further validation. In most cases, these variants correspond to ClinPGx Levels 2A and 2B annotations or their equivalent RNPGx classification, which describe moderate evidence supporting the variant-drug association. This level reflects findings supported by multiple studies but also includes a minority of studies with negative results, leading to a lower overall confidence in the association. As such, Class 2 variants are not suitable for preemptive testing and should be used for secondary or exploratory purposes only. Their further study will help validate their clinical applicability.

Looking forward, these recommendations are intended to serve as a reference for the development of decision support tools. By providing both a curated list of genes and a detailed framework for their technical analysis, this work facilitates integration into electronic medical records (EMRs), clinical decision support systems (CDS), and institutional laboratory workflows, opening up the possibility of generating pharmacogenetic alerts for clinicians when prescribing drugs [91]. It also offers a foundation for assay design by diagnostic companies and laboratories aiming to align their PGx offerings with current clinical needs and regulatory expectations.

Importantly, as genomic data increasingly originate from exome- or genome-wide sequencing, the challenge is shifting from data generation to the interpretation and clinical exploitation of relevant pharmacogenetic information. In this context, the RNPGx recommendations provide a structured reference to identify, prioritize, and extract clinically actionable variants from large-scale sequencing datasets.

5 | Conclusion

In summary, the RNPGx recommendations aim to support the harmonized deployment of pharmacogenetics in routine care by defining a core set of genes to be systematically analyzed and proposing adaptable strategies for their analysis. Their integration into decision support infrastructures and prospective validation in real-world settings represents key perspectives for advancing the clinical utility of pharmacogenetics.

Author Contributions

Nicolas Picard and Vincent Haufroid served as project leaders. Estelle Ayme-Dietrich, Jean-Christophe Boyer, Sylvie Quaranta, Nicolas Picard, and Vincent Haufroid coordinated the working group. Xavier Fonrose, Louis Lebreton, and Hugo Alarcan contributed as expert reviewers of the curated data. The first draft of the manuscript was written by Nicolas Picard with substantial contributions from Jean-Christophe Boyer, Sylvie Quaranta, Estelle Ayme-Dietrich, and Vincent Haufroid. All authors contributed to the expert evaluation of gene and variant selection, participated in the critical revision of the manuscript, and approved the final version.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. K. Litonius, N. Kulla, P. Falkenbach, et al., "Value of Pharmacogenetic Testing Assessed With Real-World Drug Utilization and Genotype Data," *Clinical Pharmacology and Therapeutics* 117, no. 1 (2025): 278–288, https://doi.org/10.1002/cpt.3458.

2. R. Mosch, M. van der Lee, H. J. Guchelaar, and J. J. Swen, "Pharmacogenetic Panel Testing: A Review of Current Practice and Potential for Clinical Implementation," *Annual Review of Pharmacology and Toxicology* 65, no. 1 (2025): 91–109, https://doi.org/10.1146/annurev-pharmtox-061724-080935.

3. J. J. Swen, C. H. van der Wouden, L. E. Manson, et al., "A 12-Gene Pharmacogenetic Panel to Prevent Adverse Drug Reactions: An Open-Label, Multicentre, Controlled, Cluster-Randomised Crossover Implementation Study," *Lancet* 401, no. 10374 (2023): 347–356, https://doi.org/10.1016/S0140-6736(22)01841-4.

4. D. W. Oslin, K. G. Lynch, M. C. Shih, et al., "Effect of Pharmacogenomic Testing for Drug-Gene Interactions on Medication Selection and Remission of Symptoms in Major Depressive Disorder: The PRIME Care Randomized Clinical Trial," *Journal of the American Medical Association* 328, no. 2 (2022): 151–161, https://doi.org/10.1001/jama.2022.9805.

5. M. C. Olson, A. Maciel, J. F. Gariepy, et al., "Clinical Impact of Pharmacogenetic-Guided Treatment for Patients Exhibiting Neuropsychiatric Disorders: A Randomized Controlled Trial," *Primary Care Companion CNS Disorders* 19, no. 2 (2017): e1–e7, https://doi.org/10.4088/PCC.16m02036.

6. H. J. Guchelaar, C. H. van der Wouden, L. E. N. Manson, et al., "Pharmacogenetic Implementation Studies-Lessons Learned From the PREPARE Study," *Clinical Pharmacology and Therapeutics* 118, no. 4 (2025): 803–812, https://doi.org/10.1002/cpt.3749.

7. N. Picard, J. C. Boyer, M. C. Etienne-Grimaldi, et al., "Pharmacogenetics-Based Personalized Therapy: Levels of Evidence and Recommendations From the French Network of Pharmacogenetics (RNPGx)," *Thérapie* 72, no. 2 (2017): 185–192, https://doi.org/10.1016/j.therap.2016.09.014.

8. E. Baudou, A. Lespine, G. Durrieu, et al., "Serious Ivermectin Toxicity and Human ABCB1 Nonsense Mutations," *New England Journal of Medicine* 383, no. 8 (2020): 787–789, https://doi.org/10.1056/NEJMc1917344.

9. J. A. Johnson, K. E. Caudle, L. Gong, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update," *Clinical Pharmacology and Therapeutics* 102, no. 3 (2017): 397–404, https://doi.org/10.1002/cpt.668.

10. C. R. King, E. Deych, P. Milligan, et al., "Gamma-Glutamyl Carboxylase and Its Influence on Warfarin Dose," *Thrombosis and Haemostasis* 104, no. 4 (2010): 750–754, https://doi.org/10.1160/TH09-11-0763.

11. R. Mahajan and A. K. Tyagi, "Pharmacogenomic Insights Into Tuberculosis Treatment Shows the NAT2 Genetic Variants Linked to Hepatotoxicity Risk: A Systematic Review and Meta-Analysis," *BMC Genomic Data* 25, no. 1 (2024): 103, https://doi.org/10.1186/s12863-024-01286-y.

12. H. de Jonge, C. Metalidis, M. Naesens, D. Lambrechts, and D. R. J. Kuypers, "The P450 Oxidoreductase *28 SNP Is Associated With Low Initial Tacrolimus Exposure and Increased Dose Requirements in CYP3A5-Expressing Renal Recipients," *Pharmacogenomics* 12, no. 9 (2011): 1281–1291, https://doi.org/10.2217/pgs.11.77.

13. Q. Mao and J. D. Unadkat, "Role of the Breast Cancer Resistance Protein (BCRP/ABCG2) in Drug Transpor—An Update," *AAPS Journal* 17, no. 1 (2015): 65–82, https://doi.org/10.1208/s12248-014-9668-6.

14. Y. Imai, M. Nakane, K. Kage, et al., "C421A Polymorphism in the Human Breast Cancer Resistance Protein Gene Is Associated With Low Expression of Q141K Protein and Low-Level Drug Resistance," *Molecular Cancer Therapeutics* 1, no. 8 (2002): 611–616.

15. Y. Toyoda, A. Mančíková, V. Krylov, et al., "Functional Characterization of Clinically-Relevant Rare Variants in ABCG2 Identified in a Gout and Hyperuricemia Cohort," *Cells* 8, no. 4 (2019): 363, https://doi.org/10.3390/cells8040363.

16. O. Lockridge, "Review of Human Butyrylcholinesterase Structure, Function, Genetic Variants, History of Use in the Clinic, and Potential Therapeutic Uses," *Pharmacology & Therapeutics* 148, (2015): 34–46, https://doi.org/10.1016/j.pharmthera.2014.11.011.

17. X. Brazzolotto, S. Courcelle, C. Sauvanet, et al., "Characterization of Four BCHE Mutations Associated With Prolonged Effect of Suxamethonium," *Pharmacogenomics Journal* 21, no. 2 (2021): 165–173, https://doi.org/10.1038/s41397-020-00192-7.

18. C. Millet, B. Plaud, and H. Delacour, "Phenotype and Genotype Characteristics of 58 Patients Showing a Prolonged Effect of Succinylcholine: A Four-Year Experience," *Aaesthesia, Critical Care and Pain Medicine* 40, no. 2 (2021): 100847, https://doi.org/10.1016/j.accpm.2021.100847.

19. Z. Desta, R. S. Gammal, L. Gong, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2B6 and Efavirenz-Containing Antiretroviral Therapy," *Clinical Pharmacology and Therapeutics* 106, no. 4 (2019): 726–733, https://doi.org/10.1002/cpt.1477.

20. C. A. Bousman, J. M. Stevenson, L. B. Ramsey, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants," *Clinical Pharmacology and Therapeutics* 114, no. 1 (2023): 51–68, https://doi.org/10.1002/cpt.2903.

21. M. A. Perera, L. H. Cavallari, N. A. Limdi, et al., "Genetic Variants Associated With Warfarin Dose in African-American Individuals: A Genome-Wide Association Study," *Lancet* 382, no. 9894 (2013): 790–796, https://doi.org/10.1016/S0140-6736(13)60681-9.

22. I. G. Asiimwe, E. J. Zhang, R. Osanlou, et al., "Genetic Factors Influencing Warfarin Dose in Black-African Patients: A Systematic Review and Meta-Analysis," *Clinical Pharmacology and Therapeutics* 107, no. 6 (2020): 1420–1433, https://doi.org/10.1002/cpt.1755.

23. L. S. Bråten, T. Haslemo, M. M. Jukic, et al., "A Novel CYP2C-Haplotype Associated With Ultrarapid Metabolism of Escitalopram," *Clinical Pharmacology and Therapeutics* 110, no. 3 (2021): 786–793, https://doi.org/10.1002/cpt.2233.

24. L. S. Bråten, M. Ingelman-Sundberg, M. M. Jukic, E. Molden, and M. K. Kringen, "Impact of the Novel CYP2C:TG Haplotype and CYP2B6 Variants on Sertraline Exposure in a Large Patient Population," *Clinical and Translational Science* 15, no. 9 (2022): 2135–2145, https://doi.org/10.1111/cts.13347.

25. P. S. Kee, S. D. S. Maggo, M. A. Kennedy, et al., "Omeprazole Treatment Failure in Gastroesophageal Reflux Disease and Genetic Variation at the CYP2C Locus," *Frontiers in Genetics* 13, (2022): 869160, https://doi.org/10.3389/fgene.2022.869160.

26. L. Ganoci, J. Palić, V. Trkulja, et al., "Is CYP2C Haplotype Relevant for Efficacy and Bleeding Risk in Clopidogrel-Treated Patients?," *Genes* 15, no. 5 (2024): 607, https://doi.org/10.3390/genes15050607.

27. V. M. Pratt, A. L. del Tredici, H. Hachad, et al., "Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology," *Journal of Molecular Diagnostics* 20, no. 3 (2018): 269–276, https://doi.org/10.1016/j.jmoldx.2018.01.011.

28. M. V. Relling and T. E. Klein, "CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network," *Clinical Pharmacology and Therapeutics* 89, no. 3 (2011): 464–467, https://doi.org/10.1038/clpt.2010.279.

29. C. R. Lee, J. A. Luzum, K. Sangkuhl, et al., "Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 Update," *Clinical Pharmacology and Therapeutics* 112, no. 5 (2022): 959–967, https://doi.org/10.1002/cpt.2526.

30. L. Lebreton, J. C. Boyer, C. Lafay-Chebassier, et al., "French-Speaking Network of Pharmacogenetics (RNPGx) Recommendations for Clinical Use of Mavacamten," *Clinical Pharmacology and Therapeutics* 117, no. 2 (2025): 387–397, https://doi.org/10.1002/cpt.3502.

31. B. Moriyama, A. O. Obeng, J. Barbarino, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP2C19 and Voriconazole Therapy," *Clinical Pharmacology and Therapeutics* 102, no. 1 (2017): 45–51, https://doi.org/10.1002/cpt.583.

32. J. J. Lima, C. D. Thomas, J. Barbarino, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C19 and Proton Pump Inhibitor Dosing," *Clinical Pharmacology and Therapeutics* 109, no. 6 (2021): 1417–1423, https://doi.org/10.1002/cpt.2015.

33. M. Takahashi, T. Saito, M. Ito, et al., "Functional Characterization of 21 CYP2C19 Allelic Variants for Clopidogrel 2-Oxidation," *Pharmacogenomics Journal* 15, no. 1 (2015): 26–32, https://doi.org/10.1038/tpj.2014.30.

34. M. R. Botton, X. Lu, G. Zhao, et al., "Structural Variation at the CYP2C Locus: Characterization of Deletion and Duplication Alleles," *Human Mutation* 40, no. 11 (2019): e37–e51, https://doi.org/10.1002/humu.23855.

35. C. R. Lee, J. A. Goldstein, and J. A. Pieper, "Cytochrome P450 2C9 Polymorphisms: A Comprehensive Review of the In-Vitro and Human Data," *Pharmacogenetics* 12, no. 3 (2002): 251–263, https://doi.org/10.1097/00008571-200204000-00010.

36. G. P. Aithal, C. P. Day, P. J. Kesteven, and A. K. Daly, "Association of Polymorphisms in the Cytochrome P450 CYP2C9 With Warfarin Dose Requirement and Risk of Bleeding Complications," *Lancet* 353, no. 9154 (1999): 717–719, https://doi.org/10.1016/S0140-6736(98)04474-2.

37. C. Taylor, I. Crosby, V. Yip, P. Maguire, M. Pirmohamed, and R. M. Turner, "A Review of the Important Role of CYP2D6 in Pharmacogenomics," *Genes* 11, no. 11 (2020): 1295, https://doi.org/10.3390/genes11111295.

38. M. Ingelman-Sundberg, M. Oscarson, and R. A. McLellan, "Polymorphic Human Cytochrome P450 Enzymes: An Opportunity for Individualized Drug Treatment," *Trends in Pharmacological Sciences* 20, no. 8 (1999): 342–349, https://doi.org/10.1016/s0165-6147(99)01363-2.

39. M. van der Lee, W. G. Allard, R. H. A. M. Vossen, et al., "Toward Predicting CYP2D6-Mediated Variable Drug Response From CYP2D6 Gene Sequencing Data," *Science Translational Medicine* 13, no. 603 (2021): eabf3637, https://doi.org/10.1126/scitranslmed.abf3637.

40. K. E. Caudle, K. Sangkuhl, M. Whirl-Carrillo, et al., "Standardizing CYP2D6 Genotype to Phenotype Translation: Consensus Recommendations From the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group," *Clinical and Translational Science* 13, no. 1 (2020): 116–124, https://doi.org/10.1111/cts.12692.

41. V. M. Pratt, L. H. Cavallari, A. L. del Tredici, et al., "Recommendations for Clinical CYP2D6 Genotyping Allele Selection: A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy," *Journal of Molecular Diagnostics* 23, no. 9 (2021): 1047–1064, https://doi.org/10.1016/j.jmoldx.2021.05.013.

42. A. Gaedigk, S. T. Casey, M. Whirl-Carrillo, N. A. Miller, and T. E. Klein, "Pharmacogene Variation Consortium: A Global Resource and Repository for Pharmacogene Variation," *Clinical Pharmacology and Therapeutics* 110, no. 3 (2021): 542–545, https://doi.org/10.1002/cpt.2321.

43. A. J. Turner, C. Nofziger, B. E. Ramey, et al., "PharmVar Tutorial on CYP2D6 Structural Variation Testing and Recommendations on Reporting," *Clinical Pharmacology and Therapeutics* 114, no. 6 (2023): 1220–1237, https://doi.org/10.1002/cpt.3044.

44. V. M. Pratt, L. H. Cavallari, M. L. Fulmer, et al., "CYP3A4 and CYP3A5 Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase," *Journal of Molecular Diagnostics* 25, no. 9 (2023): 619–629, https://doi.org/10.1016/j.jmoldx.2023.06.008.

45. D. Wang, Y. Guo, S. A. Wrighton, G. E. Cooke, and W. Sadee, "Intronic Polymorphism in CYP3A4 Affects Hepatic Expression and Response to Statin Drugs," *Pharmacogenomics Journal* 11, no. 4 (2011): 274–286, https://doi.org/10.1038/tpj.2010.28.

46. L. Beunk, M. Nijenhuis, B. Soree, et al., "Dutch Pharmacogenetics Working Group (DPWG) Guideline for the Gene-Drug Interaction Between CYP2D6, CYP3A4 and CYP1A2 and Antipsychotics," *European Journal of Human Genetics* 32, no. 3 (2024): 278–285, https://doi.org/10.1038/s41431-023-01347-3.

47. K. A. Birdwell, B. Decker, J. M. Barbarino, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing," *Clinical Pharmacology and Therapeutics* 98, no. 1 (2015): 19–24, https://doi.org/10.1002/cpt.113.

48. J. B. Woillard, L. Chouchana, N. Picard, M. A. Loriot, and French Network of Pharmacogenetics (RNPGX), "Pharmacogenetics of Immunosuppressants: State of the Art and Clinical Implementation—Recommendations From the French National Network of Pharmacogenetics (RNPGx)," *Thérapie* 72, no. 2 (2017): 285–299, https://doi.org/10.1016/j.therap.2016.09.016.

49. D. E. Stec, R. J. Roman, A. Flasch, and M. J. Rieder, "Functional Polymorphism in Human CYP4F2 Decreases 20-HETE Production," *Physiological Genomics* 30, no. 1 (2007): 74–81, https://doi.org/10.1152/physiolgenomics.00003.2007.

50. P. Borgiani, C. Ciccacci, V. Forte, et al., "CYP4F2 Genetic Variant (rs2108622) Significantly Contributes to Warfarin Dosing Variability in the Italian Population," *Pharmacogenomics* 10, no. 2 (2009): 261–266, https://doi.org/10.2217/14622416.10.2.261.

51. E. Danese, M. Montagnana, J. A. Johnson, et al., "Impact of the CYP4F2 p.V433M Polymorphism on Coumarin Dose Requirement: Systematic Review and Meta-Analysis," *Clinical Pharmacology and Therapeutics* 92, no. 6 (2012): 746–756, https://doi.org/10.1038/clpt.2012.184.

52. V. M. Pratt, L. H. Cavallari, M. L. Fulmer, et al., "DPYD Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, American College of Medical Genetics and Genomics, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, Pharmacogenomics Knowledgebase, and Pharmacogene Variation Consortium," *Journal of Molecular Diagnostics* 26, no. 10 (2024): 851–863, https://doi.org/10.1016/j.jmoldx.2024.05.015.

53. V. Boige, M. Vincent, P. Alexandre, et al., "DPYD Genotyping to Predict Adverse Events Following Treatment With Fluorouracil-Based Adjuvant Chemotherapy in Patients With Stage III Colon Cancer: A Secondary Analysis of the PETACC-8 Randomized Clinical Trial," *JAMA Oncology* 2, no. 5 (2016): 655–662, https://doi.org/10.1001/jamaoncol.2015.5392.

54. A. de Haar-Holleman, P. J. Cortoos, J. Vlaeminck, et al., "Case Report: A Case of Severe Capecitabine Toxicity due to Confirmed in Trans Compound Heterozygosity of a Common and Rare DPYD Variant," *Frontiers in Pharmacology* 15, (2024): 1459565, https://doi.org/10.3389/fphar.2024.1459565.

55. T. J. Wigle, S. Medwid, C. Ross, U. I. Schwarz, and R. B. Kim, "DPYD Exon 4 Deletion Associated With Fluoropyrimidine Toxicity and Importance of Copy Number Variation," *Current Oncology* 30, no. 1 (2023): 663–672, https://doi.org/10.3390/curroncol30010051.

56. J. I. Kiiski, S. Khan, A. Hosio, M. Neuvonen, and M. Niemi, "Genetic and Functional Characterization of DPYD Exon 4 Deletion Common in the Finnish Population," *Basic & Clinical Pharmacology & Toxicology* 136, no. 3 (2025): e70011, https://doi.org/10.1111/bcpt.70011.

57. WHO, "Meeting Report of the Technical Consultation to Review the Classification of Glucose-6-Phosphate Dehydrogenase (G6PD)," (2022), https://www.who.int/publications/m/item/WHO-UCN-GMP-MPAG-2022.01.

58. A. Minucci, K. Moradkhani, M. J. Hwang, C. Zuppi, B. Giardina, and E. Capoluongo, "Glucose-6-Phosphate Dehydrogenase (G6PD) Mutations Database: Review of the "Old" and Update of the New Mutations," *Blood Cells, Molecules & Diseases* 48, no. 3 (2012): 154–165, https://doi.org/10.1016/j.bcmd.2012.01.001.

59. R. S. Gammal, M. Pirmohamed, A. A. Somogyi, et al., "Expanded Clinical Pharmacogenetics Implementation Consortium Guideline for Medication Use in the Context of G6PD Genotype," *Clinical Pharmacology and Therapeutics* 113, no. 5 (2023): 973–985, https://doi.org/10.1002/cpt.2735.

60. M. J. Rieder, A. P. Reiner, and A. E. Rettie, "Gamma-Glutamyl Carboxylase (GGCX) tagSNPs Have Limited Utility for Predicting Warfarin Maintenance Dose," *Journal of Thrombosis and Haemostasis* 5, no. 11 (2007): 2227–2234, https://doi.org/10.1111/j.1538-7836.2007.02744.x.

61. S. Hetherington, S. McGuirk, G. Powell, et al., "Hypersensitivity Reactions During Therapy With the Nucleoside Reverse Transcriptase Inhibitor Abacavir," *Clinical Therapeutics* 23, no. 10 (2001): 1603–1614, https://doi.org/10.1016/s0149-2918(01)80132-6.

62. W. H. Chung, S. I. Hung, H. S. Hong, et al., "Medical Genetics: A Marker for Stevens-Johnson Syndrome," *Nature* 428, no. 6982 (2004): 486, https://doi.org/10.1038/428486a.

63. C. Y. Ng, Y. T. Yeh, C. W. Wang, et al., "Impact of the HLA-B(*)58:01 Allele and Renal Impairment on Allopurinol-Induced Cutaneous Adverse Reactions," *Journal of Investigative Dermatology* 136, no. 7 (2016): 1373–1381, https://doi.org/10.1016/j.jid.2016.02.808.

64. F. Zhou and X. Zhang, "Major Histocompatibility Complex and Psoriasis," *Journal of Investigative Dermatology. Symposium Proceedings* 19, no. 2 (2018): S79–S80, https://doi.org/10.1016/j.jisp.2018.09.006.

65. L. Lebreton, B. Hennart, S. Baklouti, et al., "Pharmacogénétique de l'Ototoxicité des Aminosides: état Des Connaissances et des Pratiques—Recommandations du Réseau Francophone de Pharmacogénétique (RNPGx)," *Thérapie* 79, no. 6 (2024): 709–717, https://doi.org/10.1016/j.therap.2024.05.006.

66. P. Frosst, Z. Zhang, A. Pai, and R. Rozen, "The Methylenetetrahydrofolate Reductase (Mthfr) Gene Maps to Distal Mouse Chromosome 4," *Mammalian Genome* 7, no. 11 (1996): 864–865, https://doi.org/10.1007/s003359900255.

67. K. H. van der Pol, M. Nijenhuis, B. Soree, et al., "Dutch Pharmacogenetics Working Group Guideline for the Gene-Drug Interaction of ABCG2, HLA-B and Allopurinol, and MTHFR, Folic Acid and Methotrexate," *European Journal of Human Genetics* 32, no. 2 (2024): 155–162, https://doi.org/10.1038/s41431-022-01180-0.

68. A. V. Pandey and P. Sproll, "Pharmacogenomics of Human P450 Oxidoreductase," *Frontiers in Pharmacology* 5, (2014): 103, https://doi.org/10.3389/fphar.2014.00103.

69. L. Elens, A. J. M. Nieuweboer, S. J. Clarke, et al., "Impact of POR*28 on the Clinical Pharmacokinetics of CYP3A Phenotyping Probes Midazolam and Erythromycin," *Pharmacogenetics and Genomics* 23, no. 3 (2013): 148–155, https://doi.org/10.1097/FPC.0b013e32835dc113.

70. P. J. Isackson, J. Wang, M. Zia, et al., "RYR1 and CACNA1S Genetic Variants Identified With Statin-Associated Muscle Symptoms," *Pharmacogenomics* 19, no. 16 (2018): 1235–1249, https://doi.org/10.2217/pgs-2018-0106.

71. S. G. Gonsalves, R. T. Dirksen, K. Sangkuhl, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for the Use of Potent Volatile Anesthetic Agents and Succinylcholine in the Context of RYR1 or CACNA1S Genotypes," *Clinical Pharmacology and Therapeutics* 105, no. 6 (2019): 1338–1344, https://doi.org/10.1002/cpt.1319.

72. J. J. Johnston, R. T. Dirksen, T. Girard, et al., "Updated Variant Curation Expert Panel Criteria and Pathogenicity Classifications for 251 Variants for RYR1-Related Malignant Hyperthermia Susceptibility," *Human Molecular Genetics* 31, no. 23 (2022): 4087–4093, https://doi.org/10.1093/hmg/ddac145.

73. R. G. Tirona, B. F. Leake, G. Merino, and R. B. Kim, "Polymorphisms in OATP-C: Identification of Multiple Allelic Variants Associated With

Altered Transport Activity Among European- and African-Americans," *Journal of Biological Chemistry* 276, no. 38 (2001): 35669–35675, https://doi.org/10.1074/jbc.M103792200.

74. M. Niemi, E. Schaeffeler, T. Lang, et al., "High Plasma Pravastatin Concentrations Are Associated With Single Nucleotide Polymorphisms and Haplotypes of Organic Anion Transporting Polypeptide-C (OATP-C, SLCO1B1)," *Pharmacogenetics* 14, no. 7 (2004): 429–440, https://doi.org/10.1097/01.fpc.0000114750.08559.32.

75. SEARCH Collaborative Group, E. Link, S. Parish, et al., "SLCO1B1 Variants and Statin-Induced Myopathy—A Genomewide Study," *New England Journal of Medicine* 359, no. 8 (2008): 789–799, https://doi.org/10.1056/NEJMoa0801936.

76. L. B. Ramsey, G. H. Bruun, W. Yang, et al., "Rare Versus Common Variants in Pharmacogenetics: SLCO1B1 Variation and Methotrexate Disposition," *Genome Research* 22, no. 1 (2012): 1–8, https://doi.org/10.1101/gr.129668.111.

77. E. van de Steeg, V. Stránecký, H. Hartmannová, et al., "Complete OATP1B1 and OATP1B3 Deficiency Causes Human Rotor Syndrome by Interrupting Conjugated Bilirubin Reuptake Into the Liver," *Journal of Clinical Investigation* 122, no. 2 (2012): 519–528, https://doi.org/10.1172/JCI59526.

78. M. L. Appell, J. Berg, J. Duley, et al., "Nomenclature for Alleles of the Thiopurine Methyltransferase Gene," *Pharmacogenetics and Genomics* 23, no. 4 (2013): 242–248, https://doi.org/10.1097/FPC.0b013e32835f1cc0.

79. V. M. Pratt, L. H. Cavallari, M. L. Fulmer, et al., "TPMT and NUDT15 Genotyping Recommendations: A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase," *Journal of Molecular Diagnostics* 24, no. 10 (2022): 1051–1063, https://doi.org/10.1016/j.jmoldx.2022.06.007.

80. K. Kawakami, D. Salonga, J. M. Park, et al., "Different Lengths of a Polymorphic Repeat Sequence in the Thymidylate Synthase Gene Affect Translational Efficiency but Not Its Gene Expression," *Clinical Cancer Research Official Journal of American Association for Cancer Research* 7, no. 12 (2001): 4096–4101.

81. S. Marsh, M. M. Ameyaw, J. Githang'a, A. Indalo, D. Ofori-Adjei, and H. L. McLeod, "Novel Thymidylate Synthase Enhancer Region Alleles in African Populations," *Human Mutation* 16, no. 6 (2000): 528, https://doi.org/10.1002/1098-1004(200012)16:6<528::AID-HUMU11>3.0.CO;2-W.

82. D. Schaerer, T. K. Froehlich, S. Hamzic, et al., "A Novel Nomenclature for Repeat Motifs in the Thymidylate Synthase Enhancer Region and Its Relevance for Pharmacogenetic Studies," *Journal of Personalized Medicine* 10, no. 4 (2020): 181, https://doi.org/10.3390/jpm10040181.

83. S. Hamzic, D. Kummer, T. K. Froehlich, et al., "Evaluating the Role of ENOSF1 and TYMS Variants as Predictors in Fluoropyrimidine-Related Toxicities: An IPD Meta-Analysis," *Pharmacological Research* 152, (2020): 104594, https://doi.org/10.1016/j.phrs.2019.104594.

84. M. C. Etienne-Grimaldi, J. C. Boyer, F. Thomas, et al., "UGT1A1 Genotype and Irinotecan Therapy: General Review and Implementation in Routine Practice," *Fundamental & Clinical Pharmacology* 29, no. 3 (2015): 219–237, https://doi.org/10.1111/fcp.12117.

85. E. C. Hulshof, M. J. Deenen, M. Nijenhuis, et al., "Dutch Pharmacogenetics Working Group (DPWG) Guideline for the Gene–Drug Interaction Between UGT1A1 and Irinotecan," *European Journal of Human Genetics* 31, no. 9 (2023): 982–987, https://doi.org/10.1038/s41431-022-01243-2.

86. R. S. Gammal, M. H. Court, C. E. Haidar, et al., "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for UGT1A1 and Atazanavir Prescribing," *Clinical Pharmacology and Therapeutics* 99, no. 4 (2016): 363–369, https://doi.org/10.1002/cpt.269.

87. N. A. Limdi, M. Wadelius, L. Cavallari, et al., "Warfarin Pharmacogenetics: A Single VKORC1 Polymorphism Is Predictive of Dose Across 3 Racial Groups," *Blood* 115, no. 18 (2010): 3827–3834, https://doi.org/10.1182/blood-2009-12-255992.

88. M. J. Rieder, A. P. Reiner, B. F. Gage, et al., "Effect of VKORC1 Haplotypes on Transcriptional Regulation and Warfarin Dose," *New England Journal of Medicine* 352, no. 22 (2005): 2285–2293, https://doi.org/10.1056/NEJMoa044503.

89. V. M. Pratt, L. H. Cavallari, A. L. del Tredici, et al., "Recommendations for Clinical Warfarin Genotyping Allele Selection: A Report of the Association for Molecular Pathology and the College of American Pathologists," *Journal of Molecular Diagnostics* 22, no. 7 (2020): 847–859, https://doi.org/10.1016/j.jmoldx.2020.04.204.

90. M. Watzka, C. Geisen, C. G. Bevans, et al., "Thirteen Novel VKORC1 Mutations Associated With Oral Anticoagulant Resistance: Insights Into Improved Patient Diagnosis and Treatment," *Journal of Thrombosis and Haemostasis* 9, no. 1 (2011): 109–118, https://doi.org/10.1111/j.1538-7836.2010.04095.x.

91. B. T. Hall, E. Eken, L. H. Cavallari, et al., "Implementing Pharmacogenomics Clinical Decision Support: A Comprehensive Tutorial on How to Integrate the Epic Genomics Module," *Clinical Pharmacology and Therapeutics* 117, (2025): 1670–1681, https://doi.org/10.1002/cpt.3599.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Application of gene selection criteria and gene characteristics for the core pharmacogenetic panel