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Pharmacogenomics implementation: From concept to practice

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Table of contents

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Leveraging transcriptomics for precision diagnosis: Lessons learned from cancer and sepsis

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Diagnostics require precision and predictive ability to be clinically useful. Integration of multi-omic with clinical data is crucial to our understanding of disease pathogenesis and diagnosis. However, interpretation of overwhelming amounts of information at the individual level requires sophisticated computational tools for extraction of clinically meaningful outputs. Moreover, evolution of technical and analytical methods often outpaces standardisation strategies. RNA is the most dynamic component of all -omics technologies carrying an abundance of regulatory information that is least harnessed for use in clinical diagnostics. Gene expression-based tests capture genetic and non-genetic heterogeneity and have been implemented in certain diseases. For example patients with early breast cancer are spared toxic unnecessary treatments with scores based on the expression of a set of genes (e.g., Oncotype DX). The ability of transcriptomics to portray the transcriptional status at a moment in time has also been used in diagnosis of dynamic diseases such as sepsis. Gene expression profiles identify endotypes in sepsis patients with prognostic value and a potential to discriminate between viral and bacterial infection. The application of transcriptomics for patient stratification in clinical environments and clinical trials thus holds promise. In this review, we discuss the current clinical application in the fields of cancer and infection. We use these paradigms to highlight the impediments in identifying useful diagnostic and prognostic biomarkers and propose approaches to overcome them and aid efforts towards clinical implementation.

KEYWORDS

biomarker, cancer, diagnosis, sepsis, transcriptomics

1 Introduction

Precision diagnosis recognises the individuality among patients in their clinical pathway by the simultaneous analysis of multimodal data with artificial intelligence (Kline et al., 2022). Precision molecular diagnostics also guide efficient, safe and cost-effective therapeutics (Ho et al., 2020). Oncology has been at the epicenter of these developments (Wahida et al., 2023), while precision approaches in infectious diseases at the research and clinical level may help in tackling an imminent antibiotic crisis (Cook and Wright, 2022). The importance of molecular technologies has been underlined in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic (Berber et al., 2021; Wang et al., 2021), but it also highlighted the need to increase our diagnostic capacity (McDermott et al., 2021).

There is an unprecedented abundance of heterogenous data available at the clinical (electronic health records) and molecular (-omic databases) level, but occasionally

phenotypic information is incomplete to assist interpretation of high through-put data (Haendel et al., 2018). The interrogation of DNA has been under investigation as a diagnostic modality for a few decades with increasing translation into clinical care (Pirmohamed, 2023) and measurement of protein products is common practice. For instance, a combination of gene markers and a panel of proteins in CancerSEEK (Cohen et al., 2018) and methylation of circulating tumour DNA (Jin et al., 2021) are breakthroughs in early detection of solid tumours and colorectal cancer, respectively. However, other molecular modalities of genetic information, such as RNA, have been explored to a lesser extent for clinical application.

In the era of precision medicine, misdiagnosis is still common in clinical practice. In a US national epidemiologic study, serious diagnostic errors resulting in significant harm were higher for certain conditions such as spinal abscess, aortic aneurysm and dissection and lung cancer (rate per incident case of disease: 36%, 17%, and 14%, respectively) (Newman-Toker et al., 2021). A gold standard test is widely accepted as the best available method to determine the presence of a condition, but it often lacks true 100% accuracy and it succumbs to advances in knowledge and technology (Sox et al., 2013; Porta, 2016). A "good" diagnostic test should also be scalable, cost-effective, and timely. There is no doubt that we need improved diagnostic tools to guide personalised management of patients and new technologies hold promise towards that direction (Love-Koh et al., 2018). But new advances also lead to many challenges. For instance, systems science, where coupling of the molecular world with mathematics, allows the modelling of multiple components and their interactions, has the potential to replace traditional reductionist approaches focusing on a single molecule (Hasin et al., 2017). However, such technologies generate vast amounts of raw large-scale data, which is incomprehensible if not analysed, integrated and interpreted with advanced bioinformatic methods and computational tools (Apweiler et al., 2018). Such approaches may not only lead to more precise diagnosis but will also generate accessory information that may enable better understanding of mechanistic pathways, disease processes, new biomarkers and druggable targets. The major challenge apart from interpretation is how such technologies can be implemented into clinical care (Green et al., 2020). But fortunately, there are some sentinel areas where novel diagnostics have been introduced (Cohen et al., 2018; Buus et al., 2021), and we need to learn lessons from the implementation process to enable uptake of novel diagnostics in other disease areas in the future.

This narrative review attempts to summarise the potential benefits and challenges of implementation of transcriptomic-based technologies into clinical settings. Cancer (in particular breast cancer) and sepsis are the two areas where gene expression tests have been developed from bulk RNA exploration. We use these paradigms to highlight the impediments in identifying useful diagnostic and prognostic biomarkers and propose ways to circumvent difficulties in the translational pathway.

2 The transcriptome

2.1 The basics of the transcriptome

The transcriptome is the total set of expressed RNA in a cell or population of cells at a specific time point. Mature messenger RNA (mRNA), which is the interim carrier of information between the genome and protein, is transcribed from a very small fraction (less than 2% to 3%) of cellular DNA (International Human Genome Sequencing Consortium, 2004). Multiple regulators decide the fate and character of the message passed on to form proteins through various mechanisms including alternative splicing and RNA editing (de Hoon et al., 2015; Abascal et al., 2020). The regulation of the whole machinery is extremely complex and involves long non-coding RNAs (lncRNAs), microRNAs (miRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), short interfering RNAs (siRNAs) and other transcripts. Furthermore, high throughput technologies have identified a plethora of novel RNA molecules but their involvement in various cellular activities is unclear (Pertea, 2012; Palazzo and Koonin, 2020).

RNA is the most dynamic cellular component regulating gene expression through complex processes including transcription, maturation and degradation (Cao and Grima, 2020). Transcription mostly occurs intermittently (on/off promoter switches) and the size and frequency of transcription bursts contribute to the molecular phenotype of a cell at a particular time point (Eling et al., 2019). Deterministic factors drive the mean expression of a gene without accounting for stochastic processes (Kærn et al., 2005). However, intrinsic molecular fluctuations (stochastic noise) have been linked to important processes such as cell fate, immune plasticity, ageing and cancer development. The combination of deterministic and stochastic components drives non-genetic heterogeneity which is modulated by gene-regulatory circuits and results in variability in transcript abundance across seemingly homogenous cell populations (Eling et al., 2019). Although there is an inverse correlation between mean gene expression and fluctuation, it has been recently shown that changes in transcriptional noise can initiate cell re-programming and development while mean gene expression remains stable (Desai et al., 2021). Collectively, therefore, RNA corresponds to a snapshot of the cellular state and has enormous potential for application to clinical diagnostics (Byron et al., 2016).

2.2 Technologies measuring the transcriptome

Technological advancements have enhanced our understanding of the transcriptome. Reverse transcriptase polymerase chain reaction (RT-PCR) is considered a gold standard for detecting qualitatively and quantitatively a limited number of transcripts (Dramé et al., 2020). Microarrays have revolutionised our approach to RNA measurement by using probes on a solid surface that hybridise with thousands of transcripts (Schena et al., 1995). They were recognised as a key tool in advancing personalised medicine (Shi et al., 2006), but despite 25+ years of development, their clinical utility remains limited (Piccart et al., 2021). Variation in sample preparation decreases reproducibility and background noise obscures the detection of low signal transcript expression. RNA sequencing (RNA-seq) technologies are more powerful tools as pre-defining RNA targets is not required and they have a greater dynamic range. RNA-seq allows the detection of the diversity in the transcriptome through the quantification of

known and novel transcripts regardless of their abundance, including non-coding RNA, single nucleotide variants, fusion genes and splice variants (Byron et al., 2016). Moreover, RNA-seq at the level of single cell (scRNA-seq) allows the detection of previously unexplored processes such as transcriptional noise (Eling et al., 2019; Desai et al., 2021). Although RNA-seq outperforms microarrays in assessing complex gene expression profiles, prediction of clinical endpoints is not affected by the platform (Zhang et al., 2015), and data based on microarray experiments have driven a plethora of discoveries. A caveat of whole RNA sequencing is its relatively poor ability to identify and quantify low abundance transcripts. Probe-based assays targeting genes of interest, such as RNA CaptureSeq have been developed to fill this gap along with sophisticated bioinformatic algorithms aiming to increase detectability of unknown sequences (Grioni et al., 2019).

Gene expression profiling provides an enormous amount of high-resolution data from a single experiment. The size of the human transcriptome remains debatable with the majority of it referred to as "dark matter" because its function is unknown (Kapranov and St Laurent, 2012). RNA-seq exceeds the size of the human genome by generating up to six billion short reads and their assembly into the transcriptome is a challenging task (Pertea, 2012). Complex computational algorithms are deployed at multiple stages of data analysis and require bioinformatics expertise (Kukurba and Montgomery, 2015). The analytical pipelines attempt to identify a set of informative genes to guide the elucidation of novel molecular mechanisms, the development of prognostic and predictive biomarkers and the identification of druggable targets.

2.3 Clinical utility of the transcriptome

Over 100 genetic tests for 30 conditions in the field of oncology, haematology, genetic disorders and pharmacogenetics, have received FDA approval to date. Less than ten tests are based on RNA measurement and only four utilise gene expression profiles with more than two RNA targets (FDA, 2021).

Molecular diagnostics focusing on the genome suffer from a limited ability to reflect accurately the *in vivo* variability within a condition at a particular time-point and among patients. The hallmark of acute lymphoblastic leukaemia (ALL), for instance, is numerous genetic aberrations stratifying patients into prognostic and therapeutic groups (Pui et al., 2019). However, multiple mutations identified at the genome level may not be contributing to the disease. Transcriptome sequencing characterises clinically relevant genomic alterations and variants in real time with higher sensitivity compared to whole-genome sequencing and it has been crucial in the discovery of novel subtypes and therapy tailoring (Roberts and Mullighan, 2015). Gene expression studies identified the Philadelphia chromosome-like ALL subtype and the downstream involvement of kinases guiding the use of tyrosine kinase inhibitors (TKI) (Inaba et al., 2017).

Transcriptomics is explored as a complementary method to genomic testing for precision-based treatments in cancer patients (Lee et al., 2021; Tsimberidou et al., 2022). The Worldwide Innovative Network (WIN) study to select rational therapeutics based on the analysis of matched tumour and normal biopsies in subjects with advanced malignancies (WINTHER, NCT01856296) was the first large-scale prospective clinical trial that allowed a fraction of patients with no actionable DNA alterations to have RNA-guided treatments using a novel algorithm (Rodon et al., 2019). The Individualised Therapy For Relapsed Malignancies in Childhood (INFORM) registry collects real-world clinical and multi-omic data from routine biopsies to translate them to precision treatments and inform future clinical trials (van Tilburg et al., 2021). The first trial (NCT03838042) is ongoing and investigates the combination of Nivolumab and Entinostat in children and adolescents with refractory high-risk malignancies. Stratification of patients in accordance with their tumour genetic mutation and gene expression profiles will serve for the purposes of biomarker development and to minimise unnecessary risks in patients (van Tilburg et al., 2020).

Liquid biopsy of extracellular RNA (exRNA) has been embraced as a promising tool for screening and disease monitoring purposes and as an alternative to invasive methods of diagnosis such as tissue biopsy (Heitzer et al., 2019; Zhou et al., 2020; Wu et al., 2022). Although studies investigating exRNA in clinical application are scarce, recent developments in oncology are paving the way by enabling the distinction between tumour-specific RNA and total circulating extracellular transcriptome (Vermeirssen et al., 2022; Zong et al., 2023). Moreover, analysis of intracellular RNA of circulating tumour cells and peripheral blood mononuclear cells (PBMC) has identified prognostic pathways for response to treatment in patients with metastatic castration-resistant prostate cancer (Zhang et al., 2022).

3 Cancer

3.1 Transcriptomics in early breast cancer

In breast cancer, patient stratification based on expression of tumour markers (e.g., ER, PR and HER2 in breast cancer) has guided treatment strategies for over 30 years (Cardoso et al., 2016) laying the foundation for remarkable advances in molecular diagnostics (Buus et al., 2021). Early breast cancer (Supplementary Box S1) represents a successful paradigm of the applied knowledge accrued from transcriptomics in clinical practice. Only a small proportion of patients with oestrogen receptor positive (ER+) and lymph node negative (LN-) breast cancer benefit from adjuvant chemotherapy. Unfortunately, clinicopathological features poorly characterise ER+/LN- tumours and immunohistochemical techniques cannot be relied on to make treatment decisions (Fitzgibbons et al., 2000; Eifel et al., 2001). The standard practice has been to use a combination of hormonal and chemotherapy regimens, despite evidence suggesting that around 80% of patients were overtreated and unnecessarily exposed to chemotherapy and the potential toxicity (van 't Veer et al., 2002). Hence, identification of gene expression signatures able to predict risk of recurrence, and therefore stratify treatment, was a breakthrough in early breast cancer management (Schaafsma et al., 2021).

Commercially available assays, such as Oncotype DX (Genomic Health), MammaPrint (Agendia), EndoPredict (Myriad Genetics) and Prosigna (Nanostring Technologies) are endorsed by the UK National Institute for Health and Care Excellence (NICE) and

TABLE 1 Examples of commercialised gene expression tests and their characteristics.

Disease[a]	Trade name [manufacturer] (reference)	No of genes in signature	Platform	Clinical use	Guidelines[b]
Early breast cancer	Oncotype Dx (Genomic Health, now Exact Sciences) Paik et al. (2004), Sparano et al. (2018), Syed (2020)	21 (16 cancer-related and 5 reference genes)	RT-PCR	Prognostic of 10-year distant recurrence risk and predictive of adjuvant chemotherapy benefit in HR+/HER2-/LN ≤ 3	ASCO, ESMO [I, A], NCCN (Category 1) and NICE recommendation Cardoso et al. (2019), Henry et al. (2019), National Comprehensive Cancer Network (2021a), NICE (2018a)
	MammaPrint (Agentia) Cardoso et al. (2016), Piccart et al. (2021), van 't Veer et al. (2002)	70	Microarray	Prognostic of distant recurrence in women older than 50 years with HR+/HER2-/LN ≤ 3/T ≤ 5 cm	ASCO, ESMO [I, A] and NCCN (Category 1) recommendation (NICE does not recommend as it was not found to be cost-effective) Cardoso et al. (2019), Henry et al. (2019), National Comprehensive Cancer Network (2021a), NICE (2018a)
	Endopredict (Myriad Genetics)	12 (8 cancer-related and 3 reference genes)	RT-PCR	Prognostic of 10-year distant recurrence risk in HR+/HER2-/LN ≤ 3 treated with endocrine therapy alone	ESMO [I, B], NCCN (Category 2A) and NICE recommendation Cardoso et al. (2019), National Comprehensive Cancer Network, (2021a), NICE, (2018b)
	Prosigna (NanoString Technologies)	50 (+5 reference genes)	N-Counter[c]	Prognostic of 10-year distant recurrence in postmenopausal women with ER+/HER2-/LN ≤ 3.	ESMO [I, B], NCCN (Category 2A) and NICE recommendation Cardoso et al. (2019), National Comprehensive Cancer Network (2021a), NICE (2018b)
Prostate cancer	Oncotype DX (Exact Sciences)	17	RT-PCR	Prognostic of adverse pathology and 10-year risk of metastasis	ASCO, NCCN Eggener et al. (2020), National Comprehensive Cancer Network (2021c)
	Prolaris (Myriad Genetics; a combination of a gene expression score and a clinical score)	31 cell cycle progression genes (+15 control genes)	RT-PCR	Prognostic of 10-year risk of metastatic disease and prostate cancer-specific mortality	ASCO, NCCN and NICE advice MIB65 Eggener et al. (2020), National Comprehensive Cancer Network (2021c), NICE (2016)
	Decipher (Veracyte)	22	Microarray	Prognostic of adverse pathology, 10-year risk of metastasis and 15-year risk of prostate cancer-specific mortality	ASCO, NCCN Eggener et al. (2020), National Comprehensive Cancer Network (2021c)
Colon cancer	Oncotype DX (Exact Sciences)	12 (7 cancer-related and 5 reference genes)	RT-PCR	Prognostic of recurrence in stage II and III colon cancer	Not recommended National Comprehensive Cancer Network (2021b)
	ColoPrint (Agentia)	18	Microarray	Prognostic of recurrence in stage I through III colon cancer	Not recommended National Comprehensive Cancer Network (2021b)
	ColDx (Almac Diagnostic Services)	634	Microarray	Prognostic of recurrence in stage II colon cancer	Not recommended National Comprehensive Cancer Network (2021b)
Solid tumours	Caris Molecular Intelligence (Caris Life Sciences) CARIS, (2021)	HLA genotyping (55 fusions and 3 variant transcripts mostly associated with cancer and response to certain drugs)	RNA-seq	Treatment recommendations based on a multi-level molecular (DNA, RNA and protein) profiling of locally advanced or metastatic cancer	NICE advice MIB120[d] NICE (2017)
Uveal melanoma	Decision DX-UM (Castle Biosciences) Aaberg et al. (2020)	15	RT-PCR	Predictive of 5-year metastatic risk guiding surveillance	NCCN National Comprehensive Cancer Network (2022)

[a]The searches were conducted on databases (e.g., PubMed) and websites of guideline producers (e.g., NICE), leading authorities (e.g., The Centers for Medicare and Medicaid Services) and health technology assessment agencies and the lists are non-exhaustive. Additional commercially available gene expression signatures for early breast cancer: Rotterdam signature (Veridex, Johnson & Johnson), OncoMasTR, BluePrint (Agentia), Breast Cancer Index (Biotheranostics; complements histologic grading), Mapquant DX (also known as Genomic Grade Index; Ipsogen; complements histologic grading), MammaTyper (Biontech; RT-PCR, as an alternative to immunohistochemistry for quantification of HER2, ER, PR, and marker of proliferation Ki-67 used in molecular subtyping), Curbest 95GC, Breast Ca Gene Expression Ratio (Theros H/I), BreastNext, BreastOncPX, BreastPRS, combimatrix breast cancer profile, eXagen, Invasiveness Signature, Insight DX, breast cancer profile, MammoStrat, NexCourse Breast IHC4, NuvoSelect eRx 200-Gene Assay, Randox Assay, SYMPHONY, genomic breast cancer profile, TargetPrint, TheraPrint, The 41-gene signature assay, THEROS, Breast Cancer Index. Commercially available assays for other cancers: Lung RS, Oncomine Dx Target Test (lung), ExoDx Prostate EPI-CE, Afirma (thyroid), ThyroSeq v3 Genomic Classifier, DecisionDx-Melanoma (Castle Biosciences), MYPATH, Melanoma assay (Myriad Genetics), Pigmented Lesion Assay (DermTech), MyPRS, Plus GEP70 (multiple myeloma), MMprofiler (multiple myeloma), ResponseDX (cancer of unknown origin), Pathwork Test Kit (cancer of unknown origin), Oncofocus (cancer of unknown origin), CancerTypeID (cancer of unknown origin), miRview (cancer of unknown origin), RosettaCX, cancer origin test, OneRNA (RNA-seq, based test assisting in cancer treatment selection regardless of disease site). Other commercially available assays: AlloMap (heart transplant), TruGraf (kidney transplant), Corus CAD (obstructive coronary artery disease), SGES/CardioDX (coronary artery disease), PredictSure-IBD.

[b]ESMO (level of evidence, grade of recommendation).

[c]Direct mRNA, labelling with fluorescent probes and measuring with nCounter Digital Analyser.

[d]A Medtech Innovation Briefing (MIB) is not NICE, guidance but an objective description of the technology to aid clinical decision-making.

RT-PCR, Reverse transcriptase polymerase chain reaction; HR+, Hormone Receptor-positive; HER2-, Human Epidermal growth factor Receptor 2-negative; LN ≤ 3, Lymph Node-negative or up to three-positive; T ≤ 5, Tumour size up to 5 cm; ASCO, american society of clinical oncology, ESMO, European society for medical oncology; NCCN, national comprehensive cancer network; NICE, the national institute for health and care excellence; RNA-seq, RNA, sequencing.

international guidelines (Table 1). Expression levels of specific genes are measured in tumour samples with RT-PCR (Oncotype DX) or microarrays (MammaPrint) and a prognostic score is calculated with mathematical models in order to stratify patients into risk groups (Sparano et al., 2018; Piccart et al., 2021). EndoPredict produces a score based on both transcriptional and clinical (tumour size and nodal status) features. Prosigna classifies breast cancer into subtypes and calculates a score based on gene expression, subtype, clinical parameters (tumour size and nodal status) and proliferation pathways (Paik et al., 2006). Oncotype DX is based on a 21-gene signature which is independent of clinicopathological factors (Sparano et al., 2018). It is the only multi-gene assay which is validated to predict adjuvant chemotherapy benefit in addition to prognosis (Syed, 2020).

3.2 Development of Oncotype DX

The development of Oncotype DX was a gradual process involving the use of data from large clinical studies and diligent address of issues (Supplementary Box S2). Due to the remarkable molecular diversity of breast tumours (Perou et al., 2000), numerous clinical and immunohistochemical biomarkers and their combinations had failed to guide treatment decisions (Hayes, 2000). Moreover, previous attempts to identify predictive and prognostic gene expression signatures were based on single studies which were neither standardised nor reproducible. The 21-gene signature in Oncotype DX was derived from a set of 250 genes which was selected from well-designed studies and public databases utilising microarrays (Paik et al., 2004). The 250 candidate genes were narrowed down to 21 through three independent clinical studies including almost 500 patients who received adjuvant hormonal treatment plus chemotherapy or hormonal treatment alone (Paik et al., 2003; Cobleigh et al., 2005). An algorithm was developed to produce a continuous variable, the Recurrence Score (RS) based on the expression of these genes, which is comprehensible by clinicians and stratifies patients into high and low risk groups for distant recurrence within 10 years of surgery (Paik et al., 2004). RS showed remarkable statistically significant prognostic ability and predictive ability and has been extensively validated in large prospective randomised clinical trials and real-world data from population-based registries (Paik et al., 2004; Nitz et al., 2017; Sparano et al., 2018; Syed, 2020). Further analyses of these studies have identified that pre-menopausal women would benefit from the addition of clinical factors (age, tumour size, and histologic grade) along with RS for shaping management strategies (Hunter and Longo, 2019; Sparano et al., 2019).

Oncotype DX and the Decipher Genomic Classifier (21 and 22 expressed genes, respectively) have been shown to be cost-effective approaches for guidance of treatment decisions (Lobo et al., 2017; Berdunov et al., 2022). This results from a combination of test accuracy in reducing unnecessary toxic treatments such as chemotherapy and radiation while not excluding patients from beneficial treatments (Lux et al., 2022).

Following on from the success of the oncotype Dx for early breast cancer, the Oncotype DX Genomic Prostate Score has been developed on the same principles and similar processes (Supplementary Box S3). It aims to prevent unnecessary surgery and radiation by stratifying patients into low-risk and aggressive disease. However, there are no large prospective studies to validate the prognostic performance of the assay for clinical outcomes (Eggener et al., 2019; Brooks et al., 2021). Attempts to identify a gene expression signature prognostic of prostate cancer are based on tissue samples derived from needle-core biopsies and the limited amount of tissue may be a constrain to characterise heterogeneity (Supplementary Box S3).

Disease heterogeneity is a major caveat in the design of diagnostic biomarkers. Inter-assay comparisons revealed discordance in prognostic performance of gene expression-based tests for stratification of patients with early breast cancer (Varga et al., 2019; Abdelhakam et al., 2021; Buus et al., 2021). These discrepancies may derive from the diversity in gene sets, methodology and algorithms and design of studies. Of note, there is only minor overlap of genes among predictive tests (Supplementary Table S1). Heterogeneity in gene composition reflects the variety of molecular mechanisms involved in disease progression and it may not necessarily influence prognostic ability. Currently, a prospective study is investigating the clinical validity of Curebest 95GC, a microarray-based measurement of the whole genome in tumour tissues (Naoi et al., 2021). The results are anticipated to shed light on the number of transcripts required for stratification of patients with early breast cancer. However, an increased number of genes may be a major obstacle in the development of a cost-effective marker and mechanistic studies could assist with reducing the number (Gliddon et al., 2018).

3.3 Multi-layered heterogeneity at the tissue level

Heterogeneity at the tissue level is multi-layered and not confined to the oncogenic cells. Neoplastic cancer cells are nurtured by neighbouring stromal cells comprising the tumour microenvironment (TME). A diverse community of tumour infiltrating immune cells is a major component of the stromal microenvironment exerting both beneficial and detrimental effects (Hanahan and Coussens, 2012). Growing evidence shows that quantification of the proportion of leucocyte subsets can assist in prognosis and therapy choice (Gentles et al., 2015). Traditional methods such as immunohistochemistry and flow cytometry can identify a limited number of pre-defined cell populations but fail to discriminate unknown or closely related phenotypes.

By contrast, gene expression profiling coupled with computational algorithms can characterise cell composition of complex tissues (Finotello and Trajanoski, 2018; Xu et al., 2021). Many tools have been developed based on two main methods:

- *in silico* deconvolution (CYBERSORT, TIMER, EPIC, quanTIseq, DeconRNAseq, PERT, DSA, MMAD, ssKL); and
- gene set enrichment analysis (xCell, TIminer, MCP-counter) (Finotello and Trajanoski, 2018).

Deconvolution is based on a linear model of the expression of a gene in the different cell types. Digital dissection of the tumour into the relative fractions of cell types is estimated against a library of cell-

specific expression signatures (reference signature or matrix). The matrix appears rigid considering the diversity of infiltrating immune cells (extend, type, activation status, interactions, and closely related cells) among tissues and cancer stages (Hanahan and Coussens, 2012; Newman et al., 2015). Refinement of the gene set enrichment method attempts to circumvent the issue by producing enrichment scores based on the expression levels of a set of cell-type-specific marker genes by analysing various data sources (Aran et al., 2017). However, performance is poorer in real mixtures compared to simulated mixtures and statistical significance is not reported for prediction of cell abundance (Newman et al., 2015; Aran et al., 2017). Deconvolution algorithms which simultaneously estimate relative cell fractions and produce a matrix of expression profiles have been developed (MMAD, DSA, ssKL, ssFrobenius, and deconf), but they are flawed by mathematical complexity and a limited ability to quantify a higher number of immune cells (Finotello and Trajanoski, 2018). Although several studies have tested these computational approaches in simulated samples and publicly available datasets showing good performance, evidence about their clinical validity is scarce (Desmedt et al., 2018; Newman et al., 2019; Waks et al., 2019; Craven et al., 2021).

It is unclear if gene signature enrichment and deconvolution approaches accurately portray the complexity of cellular heterogeneity in cancer samples and more work is warranted before testing in clinical settings. Definition of reference expression profiles is a fundamental caveat allowing for the identification of only a few dozens of cell types which may not reflect all heterogenic subsets in tumours. The effort should probably be on revealing hallmark phenotypes with prognostic and predictive capability in clinical settings to populate the reference matrix or marker gene-sets. For instance, the role of exhausted (increased PD-1 expression) $CD8^+$ tumour infiltrating lymphocytes is well established in melanoma, renal and non-small cell lung cancer and it has guided the use of immune check point inhibitors (ICIs) (Sade-Feldman et al., 2018; Thommen et al., 2018; Young et al., 2018; McLane et al., 2019). Tumour-associated macrophages are another interesting group of cells because of their abundance in the tumour microenvironment. Unravelling of the complex subpopulations has shown that the classical categorisation to M1 and M2 polarised macrophages is an oversimplification of their crucial role in cancer regulation (Mantovani and Longo, 2018; Duan and Luo, 2021; Xiang et al., 2021).

4 Sepsis

4.1 Dynamic heterogeneity: The sepsis paradigm

Immune response to infection is initiated by a "genomic storm" of both pro-inflammatory and anti-inflammatory cytokines expressed concomitantly (Nakamori et al., 2020). In sepsis there is acute cellular reprogramming and failure to restore balance between immune activation and suppression can present with life-threatening organ dysfunction (Singer et al., 2016; van der Poll et al., 2017). Gene expression studies have revealed remarkable heterogeneity in sepsis due to host parameters (e.g., genomic variation and co-morbidities), source of infection and stage of illness. This may explain, at least partly, the reason for the failure of numerous promising therapeutic agents in clinical trials (Marshall, 2014; Davenport et al., 2016; Peters-Sengers et al., 2022). The definition of sepsis has also been revised several times, and each definition can dramatically alter the composition of cohorts that are included in studies (Johnson et al., 2018). This can also negatively impact model development, particularly where retrospective data collection is required or data are pooled across studies (Sauer et al., 2022).

To address individual variation in the response to sepsis, a multi-layered approach, including at the molecular level, for stratification in treatment subgroups is required. As a proof-of-concept, machine learning algorithms which classify patients based on routine clinical data have been shown to accurately predict clinical outcomes and sepsis onset (Komorowski et al., 2018; Seymour et al., 2019; Fleuren et al., 2020). Machine learning is a very powerful tool for harnessing large-scale data with the aim of identifying predictive biomarkers (Zhang et al., 2021). The use of diverse methods analysing transcriptomic data in various conditions has been previously reviewed (Vadapalli et al., 2022). Appreciation of common pitfalls and focus on interpretable findings has transformed these complex computational approaches into comprehensive tools (Sidak et al., 2022; Whalen et al., 2022). However, despite our increased understanding of sepsis pathogenesis with new technologies, translation of research knowledge to improvements in clinical practice has been exceedingly difficult.

4.2 Stratification of patients with sepsis

Transcriptomic-based real-time subclassification of patients has been developed and validated in individual studies (Table 2). The Knight group investigated gene expression profiles in peripheral blood leukocytes of patients on Intensive Care Units (ICU) with faecal peritonitis and community acquired pneumonia (Davenport et al., 2016). They proposed two sepsis phenotypes associated with prognosis. Genes comprising the sepsis response signature (SRS) demonstrated significant overlap between the two sources of infection and with trauma patients, while gene expression and SRS membership changed temporally (Burnham et al., 2017). Single-cell multi-omics evaluation showed that an immature immunosuppressive population of neutrophils together with enrichment in the IL-1 pathway are the biological underpinnings of the SRS1 group who experienced increased early mortality (Kwok et al., 2022). In contrast, the immuno-competency of the SRS2 endotype was compromised by corticosteroids in a randomised clinical trial which showed an association between hydrocortisone use and higher mortality in the SRS2 group but not in the SRS1 group (Antcliffe et al., 2019). The SRS investigators upgraded their classifier to the SepstratifieR framework which can be applied to multiple infecting pathogens and data accruing from different platforms (e.g., RNA-seq and RT-qPCR). SepstratifieR utilises expression levels of signature genes, including an extended 19-gene set expected to be robust to technological variation, to align samples to a corresponding reference map and returns the SRS endotype and a severity score (SRSq). SRSq reflects immune deregulation and has the advantage of modelling patients as

TABLE 2 A summary of studies identifying gene expression signatures to classify patients with critical illness due to infection.

First author, year of publication	Study design	Condition/ Infection	Sample type	Sample size	Platform	Classifier training approach	No of DEG	Biological functions/ pathways	Patient stratification	Gene signature/ classifier
Davenport et al. (2016)	Prospective observational	CAP	Peripheral blood leukocytes	Discovery: 265 Validation: 106	Illumina Human-HT-12 version 4 Expression BeadChips	Unsupervised hierarchical cluster analysis, sparse regression variable selection	3,080	T-cell activation, cell death, apoptosis, necrosis, cytotoxicity, phagocyte movement	SRS1: immuno-compromised and high mortality and SRS2: immuno-competency and low mortality	DYRK2, CCNB1IP1, TDRD9, ZAP70, ARL14EP, MDC1, ADGRE3 (Davenport signature)
Burnham et al. (2017)	Prospective observational	Faecal peritonitis (FP)	Peripheral blood leukocytes	Discovery: 67 Validation: 53	Illumina Human-HT-12 version 4 Expression BeadChips	Unsupervised hierarchical cluster analysis, sparse regression variable selection	1,075	Cell death, apoptosis, necrosis, T-cell activation, endotoxin tolerance	SRS1, SRS2, SRS1_FP and SRS2_FP	Membership assignment based on expression of the Davenport signature, plus a new six-gene signature for FP
Cano-Gamez et al. (2022)	Prospective observational	CAP, FP and health	Peripheral blood leukocytes and whole blood	Training: 909 Test: 2,355	Microarray RNA-seq RT-PCR	Diffusion maps and random forest	7,171	innate immune pathways, glycolysis, T-cell activation	SRSq: 0–1 with lower values indicating a patient is transcriptionally closer to health and higher values indicating similarity to SRS1	Davenport genes and FBXO31, BMS1, SH3GLB1, TTC3, USP5, UBAP1, PGS1, MRPS9, THOC1, NAT10, DNAJA3, SLC25A38
Scicluna et al. (2017)	Prospective observational	Probable or definite infection	Whole blood	Discovery: 306 Validation1: 216 Validation2: 265	Affymetrix Human Genome U219 96-array plates	Hierarchical consensus clustering and random forest	9,699	PRR and cytokine signalling, adaptive immune functions, heme biosynthesis, lymphocyte signalling, antigen presentation	Mars1-4 with Mars1 having highest mortality and immunosuppression, Mars3 being low risk and Mars4 having variable mortality among the cohorts	140-gene set -> BPGM:TAP2 (Mars1) GADD45A: PCGF5 (Mars2) AHNAK:PDCD10 (Mars3) IFIT5: GLTSCR2 (Mars4)
Scicluna et al. (2015)	Prospective observational	CAP	Whole blood	Discovery: 101 Validation: 70	Affymetrix Human Genome U219 96-array plates	Differential gene expression analysis of CAP vs. no-CAP, followed by nearest shrunken centroid classification	2,459	eIF2 signalling, T-cell receptor signalling and mTOR signalling	N/A	78-gene set -> FAIM3:PLAC8
Wong et al. (2009) and Wong et al. (2011)	Prospective observational	Septic shock	Whole blood	Discovery: 98 Validation: 82	Affymetrix Human Genome U133 Plus 2.0 GeneChip	Differential gene expression analysis, unsupervised hierarchical clustering, analysis functional enrichment and K-means clustering.	6,934	Adaptive immunity and glucocorticoid receptor signalling	Subclass A, B and C with A having higher illness severity and mortality and repressed gene expression patterns	100-gene set

(Continued on following page)

10.3389/fgene.2023.1100352

TABLE 2 (*Continued*) A summary of studies identifying gene expression signatures to classify patients with critical illness due to infection.

First author, year of publication	Study design	Condition/ Infection	Sample type	Sample size	Platform	Classifier training approach	No of DEG	Biological functions/ pathways	Patient stratification	Gene signature/ classifier
Wong et al. (2015)	Retrospective and prospective observational	Septic shock	Whole blood	Discovery: 168 Validation (inter-assay): 132	NanoString nCouter	100-gene set reformulated as gene expression mosaics (GEDI) and composite variability scores	n/a	Adaptive immunity and glucocorticoid receptor signalling	Subclass A and B with A having worse outcomes and lower Gene Expression Score (GES)	100-gene set summarised as an expression mosaic, GEDI
Sweeney et al. (2015)	Meta-analysis of publicly available datasets	SIRS/trauma vs. sepsis/infection	Whole blood and buffy coat	Discovery: 9 cohorts (n = 663) Validation: 15 independent cohorts[b]	Microarrays[a]	Gene filtering by effect-size and Fisher's method using leave-one-data set-out multi-cohort analysis, followed by greedy forward search modelling	82	Downstream of IL-6 and JUN	Infection z-score derived from the geometric mean of the 11-gene set with higher scores for infected patients which peaked within 1 day of diagnosis and declined over time similarly in infected and non-infected patients	Sepsis MetaScore (SMS): CEACAM1, ZDHHC19, C9orf95, GNA15, BATF, C3AR1, KIAA1370, TGFBI, MTCH1, RPGRIP1, HLA-DPB1
Sweeney et al. (2018a)	Retrospective analysis	Bacterial sepsis	Whole blood	Discovery: 14 datasets (n = 700) Validation: 9 datasets (n = 600)	Microarrays[c]	Iterative clustering algorithm (COMMUNAL) combining K-means and consensus PAM clustering, significance analysis for microarrays (SAM), greedy forward search then multinomial logistic regression on the separation scores.	n/a	IL-1 receptor, PRR activity, complement activation, adaptive immunity and interferon signalling, platelet degranulation, glycosaminoglycan binding, coagulation cascade	Inflammopathic cluster (high mortality), Adaptive (lower mortality) and Coagulopathic (high mortality and older patients)	33-gene set
McHugh et al. (2015)		Sepsis vs. non-infective systemic inflammation	Whole blood	Discovery: 74 cases vs. 31 controls (n = 105) Validation: 5 cohorts (n = 345) from MARS	Affymetrix Human Exon 1.0 ST arrays (modified) and RT-PCR for the validation cohorts	Recursive feature elimination support vector machines and backwards elimination random forests, followed by greedy search of log gene-pair ratios	n/a	Innate immunity	SeptiScore: low values correlated with low sepsis probability (cut-off of 4)	SeptiCyte Lab: PLA2G7/ PLAC8 and CEACAM4/ LAMP1 ratios

[a]Affymetrix Human Genome U133 Plus 2.0 Array (GPL570), Illumina Human-HT-12, version 4 Expression BeadChips (GPL10558) and Illumina HumanHT-12, V3.0 expression beadchip (GPL6947).

[b]n = 218 from the Glue Grant sorted-cells cohort, n = 215 from three longitudinally sampled cohorts, n = 446 from eight cohorts comparing infection vs. health, n = 274 of a cohort comparing bacterial infection vs. autoimmune inflammation or health.

[c]GPL96, GPL570, GPL571, GPL6106, GPL6244, GPL6947, GPL10332, GPL10558, and GPL13667.

DEG, Differentially expressed genes; CAP, Community acquired pneumonia; SRS, Sepsis response signature; FP, Faecal peritonitis; RNA-seq, RNA sequencing; RT-PCR, Reverse transcriptase polymerase chain reaction; PRR, Pattern recognition receptor; Mars, Molecular diagnosis and risk stratification of sepsis; eIF2, eukaryotic initiation factor 2; mTOR, mechanistic target of rapamycin; GEDI, Gene expression dynamics inspector; SIRS, Systemic inflammatory response syndrome; IL, interleukin; COMMUNAL, Combined mapping of multiple clUsteriNg algorithms.

10.3389/fgene.2023.1100352

TABLE 3 Differences between MARS and SRS discovery cohorts.

Parameter	MARS discovery cohort (*n* = 306)	SRS discovery cohort (*n* = 265)
Demographics	Netherlands	United Kingdom
Top comorbidities	None (41%)	Respiratory insufficiency (48%) and cardiovascular compromise (45%)
Source of infection	Multiple with 42% lung and 26% abdominal	Lung
SOFA score - Shock, %	6%–35%	6%–30%
AKI	43%	20%
Length of ICU stay, days	4	7
28-day mortality	28%	21%
Sample collection	PAXgene blood RNA tubes	Leukocyte separation at bedside (LeukoLOCK)
Microarray platform	Affymetrix (49,386 probes)	Illumina (47,231 probes)

Mars, Molecular diagnosis and risk stratification of sepsis; SRS, Sepsis response signature; SOFA: Sequential organ failure assessment; AKI, Acute kidney injury; ICU, Intensive care unit.

a continuum which is a better descriptor of molecular profiles compared to classes (Cano-Gamez et al., 2022).

The Molecular Diagnosis and Risk Stratification of Sepsis (MARS) project identified four endotypes (Mars1–4) in patients with sepsis admitted to ICU in the Netherlands (Scicluna et al., 2017). Biomarkers for each endotype were derived from a 140-gene expression signature (Table 2). The authors proposed that patients classified as Mars1 were the most clinically relevant group with consistently increased mortality. Comparisons with the SRS revealed an overlap between the low-risk groups SRS2 and Mars3, but not the expected enrichment of Mars1 patients within SRS1 (Scicluna et al., 2017; Cano-Gamez et al., 2022). One explanation could be the primarily leukocyte-based training data for SepstratifieR differing from the whole blood-derived RNA used for MARS signatures. The differences could also be attributed to variation in populations utilised for classifier development, technical procedures, bioinformatics analysis and study design (Table 3). At the gene level though, similarities in differential expression and active pathways were observed. Moreover, classification of MARS patients into SRS endotypes showed that SRS1 had a higher proportion of septic shock and elevated Sequential Organ Failure Assessment (SOFA) scores, but not increased mortality, reflecting the presence of unobserved variables preventing the severe sequelae of sepsis (Cano-Gamez et al., 2022).

Paediatric patients with septic shock were categorised into three groups based on a 100-gene set microarray-derived signature (Wong et al., 2009; Wong et al., 2011). Two (A and B) of the three subclasses were identified with the use of a different platform for mRNA quantification (NanoString nCounter) which has the potential for clinical application due to its decreased turnaround time and cost (Wong et al., 2015). The authors noted that subjects in subclass B and C demonstrated similar clinical phenotypes, whereas subclass A patients had poorer outcomes. The previously reported association between mortality and corticosteroid use among patients of a specific endotype was also observed, but this time within the subclass with increased mortality (subclass A, Wong et al., 2015). Interestingly, when the Mars signature was applied to the original paediatric population, only three of the four endotypes were stably recognised and there was no association between endotype categorisation and mortality (Scicluna et al., 2017). The search for prognostic biomarkers in paediatric septic shock has led to the development of the paediatric Sepsis Biomarker Risk Model (PERSEVERE), which is a predictive tool of mortality and disease severity (Jacobs et al., 2019; Wong et al., 2019). A panel of 117 gene probes possibly associated with outcome in children with septic shock was used to select 12 genes with a protein product which had a mechanistic role in immune responses to infection and was readily measured in serum. Classification and regression tree analysis reduced the number of proteins to five and selected age among various clinical parameters as the best combination of factors to predict 28-day mortality (Wong et al., 2012). PERSEVERE incorporates the protein products of those five genes and has been tested as a predictor of sepsis-related organ dysfunction in various cohorts (Wong et al., 2016; Yehya and Wong, 2018; Stanski et al., 2020; Al Gharaibeh et al., 2022; Atreya et al., 2022). Clinical utility is yet to be decided through large prospective validation studies. Utilization of proteins identified through gene expression exploratory studies may achieve better reproducibility among cohorts, but correlation of mRNA and protein product is affected by various biological and technical parameters and clinical translation is yet to be decided.

4.3 Use of publicly available datasets and validation

The aforementioned unsupervised clustering studies (Table 2) defined novel molecular subgroups in sepsis and produced data-driven classifiers with potential for clinical implementation. Although such approaches are the foundation of precision medicine, results are often non-reproducible because they accrue in a method-specific computational manner and/or from underpowered sample sizes. The availability of high-dimensional data from various studies in public databases and meta-clustering techniques have allowed the development of transcription-based models with improved representation of disease and population heterogeneity. A large pool of bacterial sepsis transcriptomic datasets (23 datasets; n = 1,300) identified three clusters which were descriptive of underlying molecular pathways, the Inflammopathic, the Adaptive and the Coagulopathic (Table 2).

Comparisons with previously published signatures showed that the inflammopathic cluster tended to overlap with the paediatric septic shock subclass B and SRS1 and the Adaptive cluster was associated with SRS2 (Sweeney et al., 2018a). Identification of the same group of sepsis patients in independent studies with separate techniques supports the existence of molecular subtypes. The addition of a third cluster in a bigger study underscores the importance of utilising large public datasets. In a community-based approach, three independent teams built four separate models to predict mortality in sepsis using all available gene expression datasets (Sweeney et al., 2018b). Despite common data inputs, there was little overlap in predictive genes between groups due to differences in analytical approaches. Still, the model performances were broadly similar. Moreover, the combination of gene expression-based predictors with routine clinical parameters was shown to improve prognostic accuracy (Wong et al., 2014; Scicluna et al., 2017; Sweeney et al., 2018b).

The predictive performance of candidate biomarkers attempting to distinguish between the presence and absence of infection in critically ill patients has historically been suboptimal (Pierrakos and Vincent, 2010; Wacker et al., 2013). An informative biomarker consisting of a gene expression ratio has been proposed to assist in discriminating between community acquired pneumonia (CAP) and non-CAP patients, but its relatively low negative predictive value precludes it from being a stand-alone diagnostic test (Scicluna et al., 2015). Similarly, the FDA approved SeptiCyte LAB (Immunexpress, Seattle, WA), which provides a score based on the expression of four genes, is intended to be used in conjunction with clinical factors and clinical judgement to distinguish patients with sepsis from non-infective systemic inflammation within 24 h of ICU admission (McHugh et al., 2015). Different studies evaluating the discriminative power of this novel biomarker have produced conflicting results (McHugh et al., 2015; Zimmerman et al., 2017; Koster-Brouwer et al., 2018). Comparison of three scores aiming to distinguish between the presence or absence of infection in critically ill patients (FAIM3:PLAC8, SeptiCyte LAB and MetaScore or SMS) demonstrated similar performance with some superiority of the SMS (Table 2) when applied to a different cohort of patients (Sweeney and Khatri, 2017; Maslove et al., 2019). The absence of gold standard reference test dictated the use of strict criteria to define cases and controls for a supervised analytical approach for classifier development (Table 2). As a result, the discovery cohort cannot mirror the wide spectrum of heterogeneity which is inherent in sepsis patients. It is likely that leveraging of clinical and technical heterogeneity seen in larger publicly available datasets and extensive validation may help in ameliorating limitations regarding generalisability.

Transcriptomic and genomic samples are collected during most clinical trials in cancer and other diseases (NIH, 2022). Their aim is to increase our understanding of molecular mechanisms. Investigators are not obliged to submit transcriptional data deriving from interventional clinical trials to public databases unless they are presented in a publication. Hence, a plethora of interesting data may become available later or never. Clearly, it is important for investigators to deposit data from their studies in a standardised format into publicly available databases as such democratisation of data undoubtedly accelerates the pace of progress. We think that adequate progress from the translational to the clinical stage can be achieved with combination of data from different populations and to this purpose investigators should be assisted in processing their raw data early and prompted to deposit them in public databases.

4.4 Timing of sampling

Although 80% of the blood transcriptome shows differential expression in critical illness, immune responses demonstrate significant commonality leading to a remarkable overlap in expressed genes in all-cause inflammation, regardless of the presence of an infection or not (van der Poll et al., 2017). A multicohort analysis of publicly available datasets showed that there is a small proportion of distinct genes in patients with sepsis compared to patients with a non-infective critical condition in samples obtained within 48 h of admission (Sweeney et al., 2015). These findings highlight the common trajectory of the transcriptional storm that settles down during recovery underscoring the importance of time-course-based approaches (Sweeney and Wong, 2016). Gene expression signatures which predict infection have been identified in the blood of hospitalised patients up to 5 days prior to onset of symptoms and/or diagnosis (Johnson et al., 2007; Cobb et al., 2009; Sweeney et al., 2015; Yan et al., 2015; Lukaszewski et al., 2022). These findings highlight the molecular events which occur before disease symptomatology. If the immune response is not successful in clearing the pathogen(s) during this period, more robust measures are deployed leading to a transcriptional storm (Figure 1).

Tests based on gene expression thus describe “the moment in time” which has the potential for guiding targeted therapies and personalised management (van der Poll et al., 2017). However, there is no way to match the expressed molecular moment to the exact point of the disease (Figure 1) because the duration of each stage varies significantly. As an example, many groups put their efforts into identifying a classifier within 24 h of ICU admission. We may assume that this is located within the transcriptional storm space, but we cannot say whether it is in the beginning, middle, end of the curve or even within the pre-disease space. The point of symptom onset relative to the infection point potentially varies among individuals and so does presentation and admission time. Hence, despite the efforts of time-based approaches, sampling time can be defined only clinically and not objectively across the gene expression course, i.e. “one fits all” is unlikely to succeed. Challenge studies with controlled infection and longitudinal designs could shed more light on the importance of defining timing of sampling, but are complex to perform and expensive, and need to have a careful ethical framework.

4.5 Biomarkers for sepsis in the pipeline

There are few promising biomarkers currently in the pipeline. A combination of three non-overlapping signatures identified from a multi-cohort analysis (Sweeney et al., 2015; Sweeney et al., 2016; Sweeney et al., 2018b) has led to TriVerity (formerly known as InSepTM HostDxTMSepsis and Inflammatix) (Mayhew et al., 2020). This 29-gene expression-based test with a turnaround time

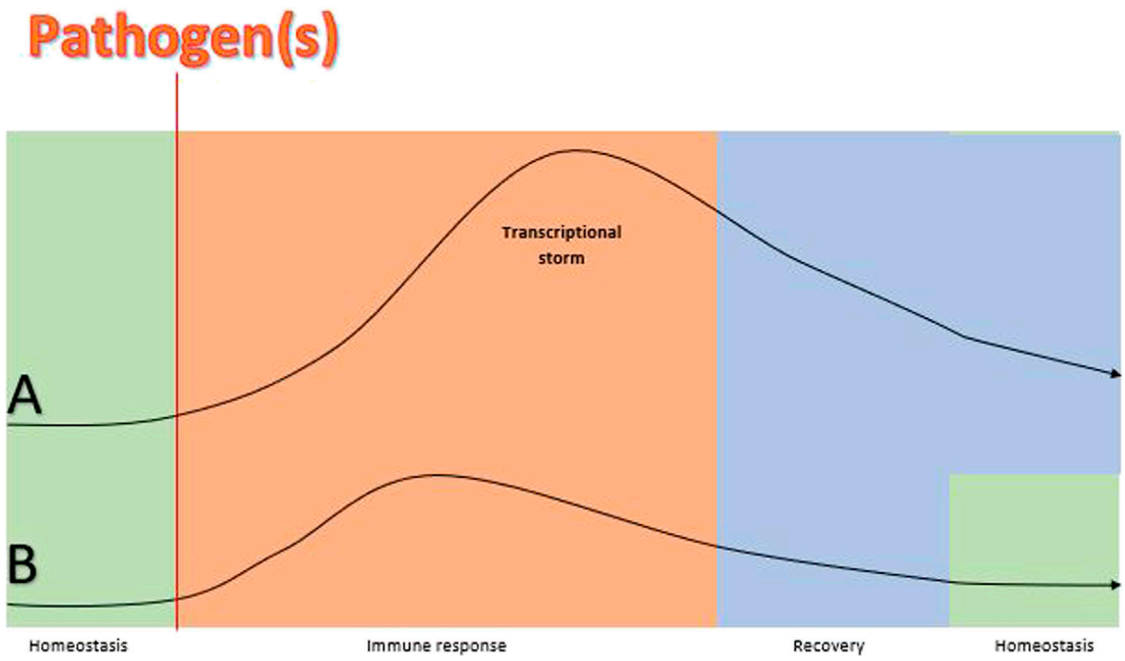

FIGURE 1
A theoretical schematic comparison of the size of gene expression trajectory before, during and after sepsis vs. gene expression response before, during and after the same infection but without sepsis. Lines **(A)** and **(B)** represent gene expression responses to a pathogen(s) in a patient with sepsis and without sepsis, respectively. The homeostasis balance (horizontal part of the lines) is disturbed in both cases by pathogen(s) but gene expression changes during the immune response phase are larger and more delayed (transcriptional storm curb) in the patient with sepsis **(A)** compared to the patient without sepsis **(B)**. The transcriptional storm represents hyper-inflammation and immunosuppression pathways which reflect the immune dysregulation in sepsis and result in organ damage (Nakamori et al., 2020). The onset of symptoms is not pointed in the diagram because the transcriptional response precedes symptomatology and this interim probably varies among individuals (Lukaszewski et al., 2022). Also, recovery is more prolonged in sepsis ans return to homeostasis may not achieved in some patients (Prescott and Angus, 2018). Findings of ongoing studies will shed light on the validity of the proposed model (Fish et al., 2022).

less than 30 min is expected to identify the presence, type (bacterial or viral) and risk of mortality of infection (Mayhew et al., 2020; Bauer et al., 2021; Safarika et al., 2021; Brakenridge et al., 2022; Galtung et al., 2022). A Point-of-Care Test claiming to distinguish bacterial from viral infections in children is in its infancy (Pennisi et al., 2021). It is based on the expression of two genes (IFI44L and FAM89A) which emerged from a microarray-based study in almost 500 febrile children (Herberg et al., 2016; Kaforou et al., 2017). There is a repertoire of promising findings in children with infections such as *tuberculosis*, bacterial pneumonia, rhinovirus and respiratory syncytial virus (RSV) and the transfer of transcriptomics knowledge to routine clinical care may be seen in the near future (Mejias et al., 2021). Investigators have also adapted a mechanistic-orientated approach to select a set of genes with known correlation with sepsis outcome instead of a crude exploration of bulk RNA (Chen et al., 2022; Kreitmann et al., 2022), but further consideration of this is beyond the scope of this review.

5 Considerations for transcriptome biomarker data analysis

The promise offered by the transcriptome in diagnosing and predicting disease status and progression is exemplified by the cancer and sepsis studies discussed. Yet relatively few RNA-based genetic tests have regulatory approval for clinical use (Table 1). This illustrates the challenges when gaining robust insights from such complex data—not least of which includes the analytical approaches that might be taken. Though the costs of sequencing continue to decrease, the number of samples in individual transcriptomic studies tend to measure in the hundreds at most, in comparison to thousands of measured RNA molecules (Levy and Myers, 2016). The chosen statistical and machine learning methods employed to produce predictive models vary greatly across studies. Table 2 provides an indication of the variety of techniques employed to classify patients, in just one clinical context. Classification approaches used include unsupervised clustering, iterative or otherwise, regression analyses, tree-based classification methods, functional enrichment and variable selection, among others. Often a combination of these methods are employed. Sweeney et al. (2018b) demonstrate this problem of choice acutely with their community-based modelling of the same data sets. Four attempts were made across three institutions to predict sepsis prognosis, yielding different models that performed similarly but had few overlapping genes. Correlations of ranked sample scores across research groups were also moderate at best. Interestingly, on average the ensemble model did not substantially differ from the individual models—suggesting some form of plateau on classification accuracy had been reached.

The choice of analysis may also be guided by the final format that the test will take in the clinic. Here the medical need, timing of the test

and costs should be considered. The proposed tests listed in Tables 1, 2 include RT-PCR assays, microarrays, Nanostring and RNA-seq methods. For sepsis where classification tests might favour rapid turnaround time, assays such as RT-PCR and Nanostring might be favourable as they yield results in a matter of hours (Wong et al., 2015). Other tests might be preferred where longer timeframes are acceptable. These might prove more cost-effective at measuring many genes, or provide robust results with convenient clinical samples such as FFPE tissue. The studies described all present a refined panel of genes or proteins as input for their classifiers, but the extent of refinement should be determined by the final assay choice for use in the clinic.

Notably, some of the attempts to apply tests to new populations find further model training is required, including the addition of more genes (Burnham et al., 2017; Cano-Gamez et al., 2022). This is perhaps to be expected given the heterogeneity of human samples and the complexity of the clinical problems. The model for Oncotype DX, approved for clinical use, was ultimately derived from pooling three clinical trials' results (Paik et al., 2014). In the case of sepsis, attempts to use publicly available data to improve robustness may similarly prove fruitful (Sweeney et al., 2018a; Sweeney et al., 2018b; Cano-Gamez et al., 2022). Likewise, more groups taking steps to ensure their analyses can be reproduced and applied to new populations, by sharing code and data, should also hasten this process (Heil et al., 2021).

Another common feature of the discussed models is their propensity for improvement by the addition or stratification of clinical variables (Sweeney et al., 2018b; Sparano et al., 2019). Where possible, routinely collected clinical variables should be incorporated early into analyses of transcriptomic data to improve the prospects of the classifier in validation studies.

Advanced machine learning methods offer the ability to flexibly model complex relationships in data. This property might be ideal when considering transcriptomics in complex clinical contexts. The flexibility may also come at a cost, in demanding greater numbers of samples than comparatively simpler methods (van der Ploeg et al., 2014). In a study comparing commonly used methods, neural network approaches failed to demonstrate superiority over regression-based analyses for classifying phenotypes from transcriptomic data (Smith et al., 2020). Another benefit of relatively parsimonious models lies in the abundance of established theory for calculating prospective study sample sizes (Riley et al., 2020). Prospective validation of a final model is essential for regulatory approval, and careful planning with realistic expectations of model performance is essential to improve the chance of success. Finally, many of the studies discussed focus on the discriminative ability of their classifiers, but lack any calibration measures for the predicted probabilities these models often estimate. These measures are vital if the models are to be used for clinical decision making (Van Calster et al., 2019). Aiming for good calibration as well as discrimination will also reduce the risk of model overfitting, thereby increasing the likelihood of prospective validation.

6 Discussion

The principles of traditional medicine should be upgraded to the tailored approaches of precision medicine. Gene expression-based tests are raw tools with a potential to be strategic for the diagnosis and management of patients. The transcriptome carries a massive amount of genetic and non-genetic information in time capturing cell, tissue, disease and host heterogeneity. The identification of transcriptional

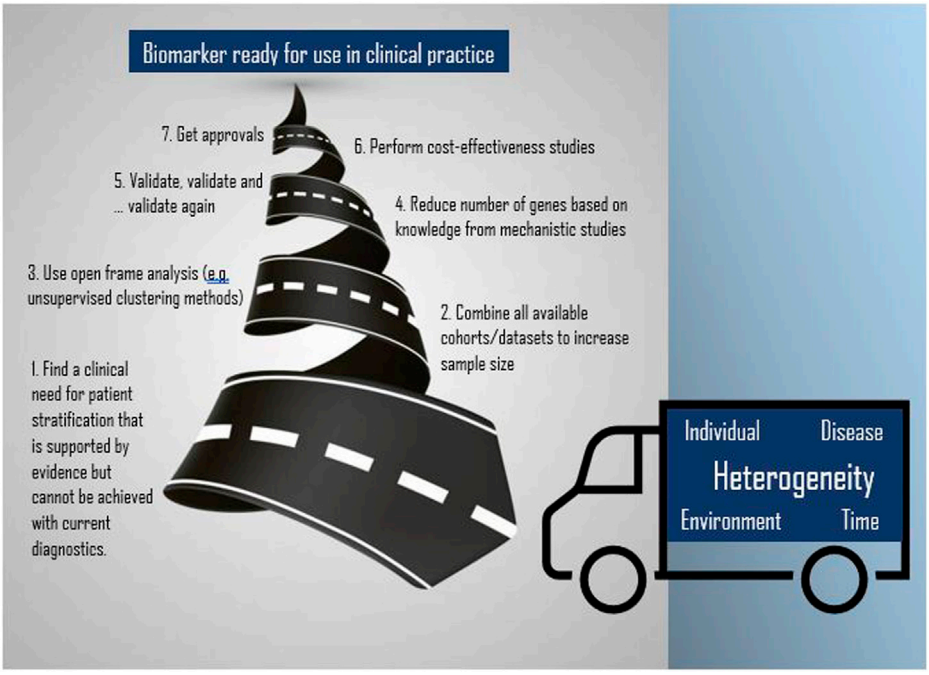

FIGURE 2
The road to implementing transcriptomics for biomarker development (spiral road image has been adapted from Vector: 13812147, standard licence reference No: 43565764).

changes which initiate cell reprogramming carry fundamental prognostic and predictive value in cancer and sepsis diagnoses. The enormous pace of evolvement of technological and analytical methods precludes standardisation and increases variation which can be circumvented with the use of large amounts of data including those which are publicly available. The accruing plethora of data, not only from a single experiment, but also from the combination of multi-cohorts, instigates the use of open-frame approaches (e.g., unsupervised hierarchical clustering) and complex mathematical algorithms resulting in computational chaos. Hence, findings require vigorous confirmation with the use of conventional methods to monitor (e.g., reference genes) processes or validate results technically and clinically. To this point, study design is paramount. Discovery studies should aim to address specific and clinically relevant questions with patient stratification into prognostic and/or treatment groups through novel diagnostic tools which outperform standard practice. Validation should be driven by large prospective randomised clinical trials and population-based studies. Our increasing knowledge of the properties of the transcriptome and its regulators is our ally in all steps of the journey of developing improved diagnostic tools (Figure 2). Breast cancer and sepsis represent exemplars for the successful development of prognostic/predictive transcriptomics-based tests underscoring the optimisation of identified gene expression signatures into clinically relevant and feasible tests. Further development in both cancer and sepsis, and indeed in other disease areas, should herald a new era of clinical diagnostics and therapeutics.

Author contributions

MP and MT contributed to the conception of the review. MT wrote the original draft and produced the tables, figures and Supplementary Material. AE wrote a section about transcriptomics analysis. MP supervised, corrected all versions and acquired funding. MT, AE, and MP contributed to manuscript revision editing and approved the submitted version.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1100352/full#supplementary-material

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Ten-year experience with pharmacogenetic testing for *DPYD* in a national cancer center in Italy: Lessons learned on the path to implementation

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Background: Awareness about the importance of implementing *DPYD* pharmacogenetics in clinical practice to prevent severe side effects related to the use of fluoropyrimidines has been raised over the years. Since 2012 at the National Cancer Institute, CRO-Aviano (Italy), a diagnostic *DPYD* genotyping service was set up.

Purpose: This study aims to describe the evolution of *DPYD* diagnostic activity at our center over the last 10 years as a case example of a successful introduction of pharmacogenetic testing in clinical practice.

Methods: Data related to the diagnostic activity of in–and out-patients referred to our service between January 2012 and December 2022 were retrieved from the hospital database.

Results: *DPYD* diagnostic activity at our center has greatly evolved over the years, shifting gradually from a post-toxicity to a pre-treatment approach. Development of pharmacogenetic guidelines by national and international consortia, genotyping, and IT technology evolution have impacted *DPYD* testing uptake in the clinics. Our participation in a large prospective implementation study (Ubiquitous Pharmacogenomics) increased health practitioners' and patients' awareness of pharmacogenetic matters and provided additional standardized infrastructures for genotyping and reporting. Nationwide test reimbursement together with recommendations by regulatory agencies in Europe and Italy in 2020 definitely changed the clinical practice guidelines of fluoropyrimidines prescription. A dramatic increase in the number of pre-treatment *DPYD* genotyping and in the coverage of new fluoropyrimidine prescriptions was noticed by the last year of observation (2022).

Conclusion: The long path to a successful *DPYD* testing implementation in the clinical practice of a National Cancer Center in Italy demonstrated that the development of pharmacogenetic guidelines and genotyping infrastructure standardization as well as capillary training and education activity for all the potential stakeholders are fundamental. However, only national health politics of test reimbursement and clear recommendations by drug regulatory agencies will definitely move the field forward.

KEYWORDS

pharmacogenetics, *DPYD*, implementation, genotyping, phenotyping, CDSS

1 Introduction

Despite the introduction of several innovative drugs in cancer treatment, fluoropyrimidines (fluorouracil and capecitabine) remain the backbone of systemic chemotherapies for a broad spectrum of solid tumors (Cavanna et al., 2006; Fernández-Martos et al., 2012; Bar-Ad et al., 2014; Heinemann et al., 2021). However, severe hematological and gastrointestinal toxicities occur in up to 30% of patients receiving fluoropyrimidines (van Kuilenburg et al., 2000; van Kuilenburg et al. 2010; van Kuilenburg, 2004; Amstutz et al., 2009; Meulendijks et al., 2015; Barin-Le Guellec et al., 2020; Sharma et al., 2021). The main fluoropyrimidines metabolizing enzyme is dihydropyrimidine dehydrogenase (DPD) representing the bottleneck in their detoxification pathway. Patients with decreased DPD activity are at risk of developing severe toxicity due to accumulation of fluoropyrimidines' active metabolites. The presence of specific variants in the coding gene (*DPYD*) has been associated with DPD deficiency and is thus predictive of an increased risk of severe side effects (Terrazzino et al., 2013; Toffoli et al., 2015; Dalle Fratte et al., 2018; Henricks et al., 2018) and associated costs (Fragoulakis et al., 2019; Toffoli et al., 2019).

International authoritative consortia, including the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG), have developed clinical pharmacogenetic (PGx) guidelines for fluoropyrimidines based on *DPYD* genotype in the clinical practice (Swen et al., 2011; Caudle et al., 2014; Amstutz et al., 2018; Bank et al., 2018; Lunenburg et al., 2020; Abdullah-Koolmees et al., 2021). In their most recent versions, both the CPIC and DPWG guidelines pointed out the importance of testing patients for the four genetic variants *DPYD*2A* (rs3918290), *DPYD*13* (rs55886062), *DPYD* c.2846A>T (rs67373798), and *DPYD* c.1236G>A (rs56038477, tagging *DPYD-HapB3*) prior to treatment with fluoropyrimidines. In 2015, a joint committee of the Italian Society of Pharmacology (SIF) and the Italian Association of Medical Oncologists (AIOM) published the first version of their own PGx guidelines specifically addressing the gene-drug interaction of *DPYD* and fluoropyrimidines (SIF-AIOM, 2015; Gori et al., 2019).

Despite the guidelines availability, implementation in clinical practice has long been delayed due to many barriers, including the lack of appropriate genotyping and Information Technology (IT) platforms (Samwald et al., 2016), reimbursement issues, and low awareness of PGx among stakeholders (Just et al., 2017). Over the years, many initiatives have been undertaken to translate PGx results into the clinical practice. In this context, the European Union funded the Ubiquitous-Pharmacogenomics (U-PGx) study, which tested the implementation of PGx guidelines at 7 clinical sites in Europe within a prospective randomized clinical trial (PREemptive Pharmacogenomic testing for prevention of Adverse drug Reactions–PREPARE) (Manson et al., 2017; Swen et al., 2023). Our institute participated in the project as the only Italian implementation site, enrolling mainly oncology patients treated with fluoropyrimidines between 2017 and early 2020 (Cecchin et al., 2017; van der Wouden et al., 2017; Blagec et al., 2018; van der Wouden et al., 2020).

Driven by large prospective studies (Henricks et al., 2018), the attention of regulatory agencies on the predictive effect of DPD tests has increased over the years, prompting the European Medicines Agency (EMA) to publish recommendations in 2020 to improve appropriateness of fluoropyrimidine use (EMA, 2020). Later, in the same year, a similar recommendation was disseminated by the Italian Regulatory Agency (AIFA) to all Italian health centres (Italian Drug Agency, 2020).

The aim of this study is to describe how *DPYD* testing at the National Cancer Institute - Centro di Riferimento Oncologico (CRO) of Aviano has evolved over the last 10 years from a spontaneous research initiative to a structured diagnostic service. We describe how adopted PGx guidelines, genotyping technologies, and physicians' awareness have changed over time. We also aimed to show how participation in the U-PGx implementation study and the publication of recommendations by European and Italian regulatory authorities have affected the *DPYD* diagnostic process in our center.

2 Materials and methods

The data analyzed in the present study were obtained from the internal database of the Experimental and Clinical Pharmacology of CRO -Aviano. It collects basic information on all the patients' derived samples entering the pharmacogenetic diagnostics and is constantly updated by the staff involved in the diagnostic process. Eligible patients were inpatients and outpatients referred to Experimental and Clinical Pharmacology for *DPYD* testing between January 2012 and December 2022. In addition, data on the number of yearly fluoropyrimidine prescriptions were collected from the hospital pharmacy database to calculate the fraction of patients with a *DPYD* test prescription each year. Data collected included: demographic information (date of birth and sex), type of biological specimen (blood or saliva) and corresponding date of specimen collection, date of specimen receipt at the laboratory, materials stored for analysis (whole blood, buffy coat, plasma, or DNA), the reason for referral and genetic results generated for reporting.

Technical details of the adopted *DPYD* genotyping panels, as well as PGx guidelines and genotyping methods introduced over the years, were retrieved from laboratory registries to describe the gradual evolution of the *DPYD* testing service. Documents and correspondence with staff and physicians involved in the U-PGx project and the PREPARE protocol and associated standard operating procedures (SOP) were reviewed to describe the standardization process of laboratory procedures. In addition, documents and certificates related to ISO 15189 and external quality assessment were consulted for analysis. Based on the information collected, a descriptive data analysis was performed to outline the evolution of PGx diagnostic activity over the past decade.

3 Results

3.1 *DPYD* diagnostic service flow at experimental and clinical pharmacology CRO-Aviano over the years

Since 2012, the Experimental and Clinical Pharmacology Unit at CRO-Aviano has offered physicians genetic testing for *DPYD* polymorphisms in patients treated with fluoropyrimidines. Over the decade under consideration, this service has evolved considerably in line with the publication of literature evidence and corresponding PGx guidelines to include an increasing number of *DPYD* variants with an expected clinical impact.

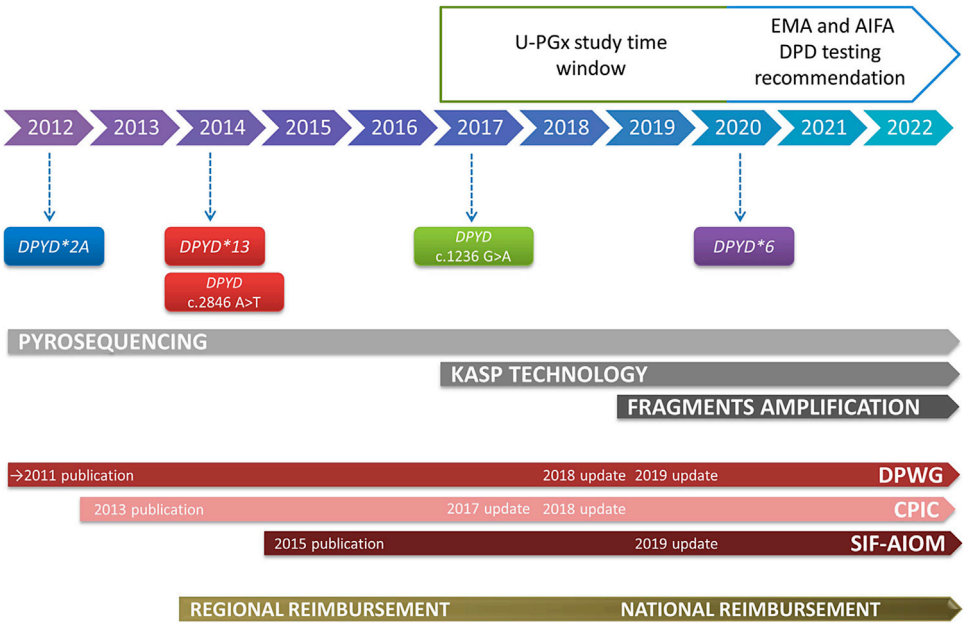

FIGURE 1
Timeline representing the evolution over the years of the *DPYD* panel tested, the genotyping technologies, the PGx guidelines adopted, and the test reimbursement at our center. The timeframe of participation to U-PGx project and publication of EMA and AIFA *DPYD* testing recommendation are highlighted. U-PGx, Ubiquitous Pharmacogenomics. EMA, European Medicines Agency. AIFA, Agenzia Italiana del Farmaco. DPD, DPYD, Di-hydroPYrimidine Dehydrogenase. DPWG, Dutch Pharmacogenetics Working Group. CPIC, Clinical Pharmacogenetics Implementation Consortium. SIF-AIOM, Società Italiana di Farmacologia- Associazione Italiana di Oncologia Medica. KASP, Kompetitive Allele Specific Polymerase chain reaction.

Initially, *DPYD* testing was performed as part of the Institute's translational pharmacogenetic research activities. Requests from prescribing oncologists were forwarded to the laboratory by telephone. After genotyping of the patient samples, the genetic results were directly reported to the requesting oncologist, who manually entered the results into the patient's medical record.

After reimbursement for *DPYD* genetic analysis was approved at the regional level since 2014, the test was formally included in our center's diagnostic services. The test prescription was electronically delivered to the laboratory by the prescribing physicians and an electronic diagnostic report was returned to the prescribing oncologist and stored in each patient's electronic clinical folder. The *DPYD* diagnostic service was made available not only to CRO-Aviano patients but also to patients from other Institutes/Regions in Italy. Since 2020 and after publication of *DPYD* testing recommendation by AIFA, the *DPYD* genotyping prescription became widespread among physicians and reimbursed by the National Health System throughout the Italian territory. This also affected the number of prescriptions in our center.

3.2 *DPYD* variants and PGx guidelines over the years

The *DPYD* genotyping panel and related recommendation have changed over the years (Figure 1).

In 2012, our laboratory started testing the *DPYD*2A* variant and adopted the 2011 DPWG guidelines of the Royal Dutch Pharmacists Association (Swen et al., 2011). The guideline recommended a 50% dose reduction in the presence of the *DPYD*2A*, variant allele or an alternative drug for carriers of two *DPYD*2A* variant alleles.

Since January 2014, *DPYD*13* and *DPYD* c.2846A>T variants were added to the panel (Swen et al., 2011; Caudle et al., 2013). Accordingly, a 50% dose reduction was recommended for heterozygous carriers of *DPYD*13* or *DPYD* c.2846A>T variant allele, as well as an alternative drug in the presence of two alleles among the two genetic polymorphisms considered.

In 2015, the collaboration between the Italian Association of Medical Oncology (AIOM) and the clinical Italian Society of Pharmacology (SIF) led to the publication of the Italian national recommendations for PGx analysis of *DPYD* in patients receiving fluoropyrimidines (SIF-AIOM, 2015), which were also considered in our laboratory as a reference for the *DPYD* diagnostic service. This first version of the Italian guidelines recommended *DPYD* testing for variants **2A*, **13*, and c.2846A>T regardless of a post-toxicity or pre-treatment approach. In particular, a 50% dose reduction was recommended for heterozygous carriers of any of these three variants, in line with international PGx guidelines (SIF-AIOM, 2015).

In 2017, we joined the European consortium U-PGx (www.upgx.eu) (van der Wouden et al., 2020; Swen, 2022) and participated in the clinical trial PREPARE (NCT03093818) a prospective, randomized European clinical trial aimed at evaluating the implementation of preemptive testing of a PGx panel, including *DPYD* for fluoropyrimidines (Swen et al., 2023). Based on the study protocol, we adopted the DPWG guidelines revised for the project purpose and made publicly available in 2018 (DPWG, 2018) for our diagnostic service. Accordingly, a fourth variant, *DPYD* 1236G>A

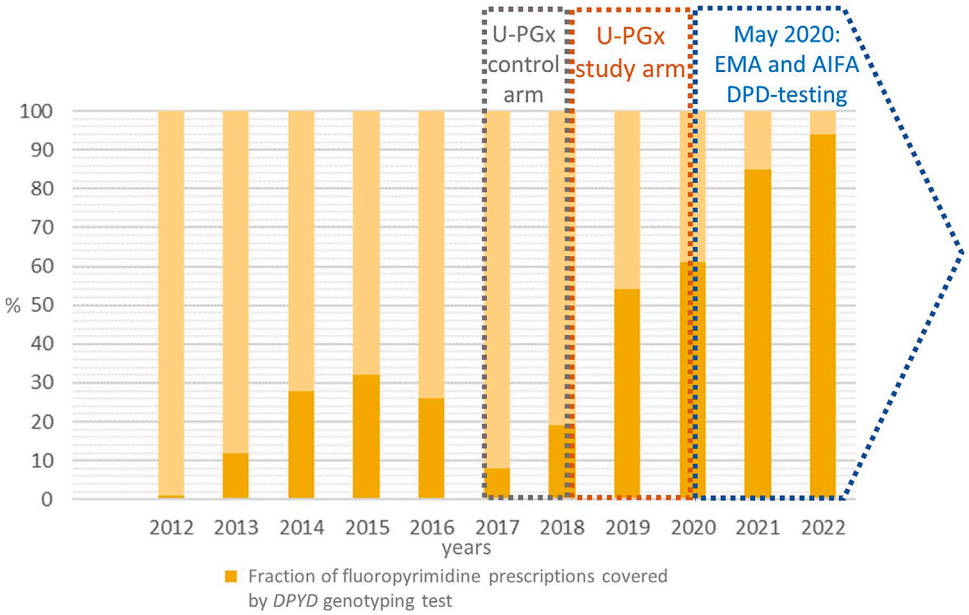

FIGURE 2
The figure reports the fraction (in percentage) of the fluoropyrimidines prescription at our center that were associated to a *DPYD* test prescription over the years. The timeframe of participation to U-PGx project (control and study arm) as well as the publication of EMA and AIFA *DPYD* testing recommendation are highlighted. U-PGx, Ubiquitous Pharmacogenomics. EMA, European Medicines Agency. AIFA, Agenzia Italiana del FArmaco. DPD, DPYD, Di-hydroPYrimidine Dehydrogenase

(rs56038477, also known as tagging *DPYD*-HapB3) was added to the panel. In addition, the concept of the *DPYD* Gene Activity Score (GAS) was introduced for fluoropyrimidine dosing recommendations, consisting of a cumulative score (0–2) to assign a toxicity risk value to different combinations of *DPYD* genotypes (see Table 1). Consistent with the project objectives, several meetings were initially organized with prescribing physicians from different hospital departments to familiarize them with the study protocol and the potential of PGx in their practice and with this approach to drug prescribing.

In November 2018, following the publication of a large prospective study testing the application of DPWG guidelines in the clinical practice (Henricks et al., 2018), CPIC published an update of their own guidelines (CPIC, 2018) suggesting that all carriers of a variant allele for one of the four variants, regardless the polymorphism, should receive a 50% dose reduction from the full standard starting dose. Accordingly, DPWG guidelines were also revised in August 2019 (DPWG, 2019; Lunenburg et al., 2020). Since 2019 we also adopted the DPWG guidelines revised version (Table 1) integrated with the Italian National guidelines SIF-AIOM (Gori et al., 2019).

According to the most updated version of the Italian National guidelines SIF-AIOM, published in October 2019, we introduced the test for *DPYD*6* (*DPYD* 2194G>A, rs1801160) (Gori et al., 2019). This additional variant should only be tested in a post-toxicity setting if the patient experienced severe toxicity after starting fluoropyrimidine treatment. In the case of a heterozygous variant allele, a 15% dose reduction is recommended, increasing to 30% if the homozygous mutant allele (*DPYD*6*/*6) is present (Gori et al., 2019).

In the last 2019 version of DPWG guidelines a new category of GAS was introduced, called "PHENO," which stands for "phenotyping". In patients with the "PHENO" GAS, genetic testing for *DPYD* is in fact deemed not sufficient to determine the initial dose reduction, and measurement of residual enzymatic activity (phenotype) is suggested. Currently, our DPD testing service does not include phenotypic analysis of the enzyme.

On 30 April 2020, the publication of the EMA recommendations (EMA, 2020) on DPD testing represented a major driver for the implementation of pre-treatment PGx in our hospital and determined the general acceptance of *DPYD* testing before the administration of fluoropyrimidines. The European Directive was implemented at the national level by AIFA on 25 May 2020 (aifa.gov, 2020). This marked the final transition from a post-toxicity to a pre-treatment approach to *DPYD* testing requests from oncologists in Italy.

3.3 Evolution of the *DPYD* genotyping platform over the years

The genotyping technologies adopted by our laboratory have changed over the years (Figure 1). Since 2012, we have used pyrosequencing technology, a mini sequencing of a fragment containing the polymorphism of interest (PSQ48, Qiagen), to perform homemade tests for genetic variants of *DPYD*.

Since 2017, we have implemented a second technology based on end-point allele specific fluorescence detection. The method was implemented in the laboratory as part of the U-PGx study. As part of the patient journey in the study, a harmonized workflow was

TABLE 1 Comparison between 2018-updated and 2019-updated versions of DPWG guidelines based on *DPYD* Gene Activity Score (DPWG, 2018; DPWG, 2019; Lunenburg et al., 2020).

2018			2019		
GAS	**Diplotype**	**Recommendation**	**GAS**	**Diplotype**	**Recommendation**
2	*1/*1	Standard dose of FPs	**2**	*1/*1	Standard dose of FPs
1.5	*1/c.1236G>A, *1/c.2846A>T	Start with 75% of the standard dose or choose an alternative	**1.5**	*1/c.2846A>T *1/c.1236G>A	Start with 50% of the standard dose or avoid FPs
1	*1/*2A	Start with 50% of the standard dose or choose an alternative	**1**	*1/*2A	Start with 50% of the standard dose or avoid FPs
	*1/*13			*1/*13	
	c.2846A>T/c.2846A>T c.1236G>A/c.1236G>A		**PHENO**	c.2846A>T/c.2846A>T c.1236G>A/c.1236G>A	[a]Determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose based on phenotype and genotype or avoid FPs
	c.2846A>T/c.1236G>A			c.2846A>T/c.1236G>A	
0.5	*2A/c.2846A>T	Start with 25% of the standard dose or choose an alternative		*2A/c.2846A>T *13/c.2846A>T *2A/c.1236G>A *13/c.1236G>A	
	*2A/c.1236G>A				
	*13/c.2846A>T				
	*13/c.1236G>A				
0	*2A/*2A	Choose an alternative. If an alternative is not possible: determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose accordingly	**0**	*2A/*2A	Avoid fluorouracil and capecitabine or determine the residual DPD activity
	*13/*13			*13/*13	
	*2A/*13			*2A/*13	

PHENO: phenotyping; GAS: gene activity score; FPs: fluoropyrimidines.
[a]DPD, enzyme activity cannot be predicted by genotype.

implemented to standardize laboratory practices and meet the requirements of the study protocol that pharmacogenetic results must be returned to prescribing physicians within three working days. The new workflow introduced in the U-PGx project was based on the use of the SNPline platform (LGC genomics, UK) using a Kompetitive Allele Specific Polymerase chain reaction (KASP) technology (van der Wouden et al., 2020).

A third method based on allele specific fragment amplification is available in the laboratory and has been used for the four polymorphisms in *DPYD* since December 2019.

Our diagnostic workflow includes independent validation of results using any two of these available methods. Concerning the analysis turnaround time, it is related to the optimization of the entire workflow, which includes blood collection and processing, reception of the analysis, DNA extraction, double genotyping procedure (using two independent methods), data analysis, and preparation of the clinical report. We noted that the use of any two of the three available methods does not affect the turnaround time of the entire process, which is now set at 3 days. Considering that samples are pooled and analyzed once a week, the maximum turnaround time is 1 week.

Since 2020, our laboratory has been undergoing an accreditation program in accordance with the International Standard ISO 15189 ("Medical laboratories–Requirements for quality and competence"), which is specifically tailored to the activities of medical laboratories and covers both the requirements for the quality system and the competence of laboratory personnel. Since 2019, the laboratory also participates in the External Quality Assessment (EQA) for laboratories delivering pharmacogenetic diagnostic tests offered by the European Molecular Genetics Quality Network (EMQN) (emqn.org).

3.4 IT genetic data management

Since 2014, *DPYD* test prescriptions and related reports have been incorporated into the existing Regional digitalized molecular diagnostic tests prescribing and reporting system. Once the oncologist prescribes a *DPYD* test in the hospital management system, blood/saliva sample collection labels are automatically generated with a unique code to track the sample sent to the laboratory.

After analysis, a genetic report is generated via the laboratory system IT DNLAB® indicating the type of biological material from which the DNA was extracted, the method used for genetic analysis, the genetic results of the *DPYD* variants analyzed, and the appropriate dosing recommendations. The report is technically and clinically validated, digitally signed, and stored as a. pdf file in the patient's electronic health record. This approach has been limited by the lack of an interactive clinical decision support system (CDSS) that could improve the application of PGx guidelines in clinical practice.

To bridge the gap, the FARMAPRICE project was launched in 2017, funded by POR FESR 2014-2020, to develop a prototype CDSS to help physicians manage their patients' genetic data and translate them into precise prescribing indications (Roncato et al., 2019). This prototype will

TABLE 2 Yearly trend of *DPYD* genotyping prescriptions referred to the Experimental and Clinical Pharmacology Unit at National Cancer Institute CRO-Aviano and the fraction of fluoropyrimidines prescriptions covered by the test.

Year	Total *DPYD* test (n)	Internal FPs prescriptions (n)	Inpatients *DPYD* test (n)	Inpatients *DPYD* test coverage (%)	Pre-treatment *DPYD* genotyping requests	
					n	%
2012	10	500	7	1	9	90
2013	49	414	49	12	49	100
2014[a]	114	408	114	28	113	99.1
2015	131	402	127	32	115	87.8
2016	111	399	102	26	95	85.6
2017	37	405	33	8	30	81
2018	94	474	88	19	89	94.7
2019	299	534	287	54	295	98.6
2020[b]	299	479	290	61	297	99.3
2021	436	463	395	85	436	100
2022	407	420	393	94	407	100

[a]Introduction of regional reimbursement.
[b]Nationwide coverage of test reimbursement. Pts: patients; FPs: fluoropyrimidines.

help physicians make safe and appropriate prescriptions. When a drug to be prescribed is entered into the FARMAPRICE platform, the physician assesses the presence of specific and validated gene-drug interactions that highlight the presence of a potentially actionable genotype for the patient by matching the PGx guideline repository with the genetic repository. If an actionable genotype is found, the physician receives a PGx-based recommendation with the appropriate level of evidence. The FARMAPRICE prototype is a ready-to-use platform that can be integrated into the hospital management system. However, implementation has been delayed due to the outbreak of COVID-19 and is pending at the time of writing.

The participation in the PREPARE study allowed us to use new IT solutions for genetic data reporting, including the use of a Genetic Information Management Platform (GIMS). GIMS provided standardized diagnostic reports including detailed genetic recommendations based on DPWG guidelines, which were constantly updated (Blagec et al., 2022). In addition, the U-PGx project provided patients with a Safety Code Card (SCC) that digitally contained their pharmacogenetic profile. The SCC was a user-friendly tool that allowed the report to be accessed in detail and in digital form via the QR code scan, so that patients and healthcare professionals could access it at any time via a smartphone.

3.5 Diagnostic activity trend over the years

During the reference period, 1,987 *DPYD* test requests were referred to the Experimental and Clinical Pharmacology Unit at National Cancer Institute CRO-Aviano. Out of the 1,987 patients, 974 (49.1%) were female and 1,013 (50.9%) were male, with mean age of 64.8 and 65.1 years, respectively. Almost 95% (1,885 samples) were inpatients, while 5.2% (102 samples) were patients from outside the hospital. Most of the collected samples were blood samples (1,855; 93.3%) and only 6.7% (132 samples) were saliva samples.

The number of inpatients receiving a fluoropyrimidine prescription each year was retrieved from the hospital pharmacy (patients who were already tested for *DPYD* variants were excluded from the count) and was compared to the number of *DPYD* tests delivered for inpatients each year. As reported in Table 2 and Figure 2 the percentage of tested inpatients increased over the years reaching 94% in 2022.

The number of patients referred for post-toxicity testing totaled 52 (2.6%), whereas the number of samples referred for pre-treatment genotyping was 1,935 (97.4%). A progressive increase in the rate of pre-treatment versus post-toxicity testing was observed over the years (Table 2).

Table 2 highlights also the trend of patients' inclusion in the *DPYD* diagnostic program in our center between January 2012 and December 2022. In the 2017-2018 time window, the number of yearly requests remained stable or slightly decreasing, due to the center's participation in the standard-of-care arm of the PREPARE clinical trial. After the switch to the PREPARE study arm in October 2018 (Swen et al., 2023) and the publication of the DPD test recommendation by EMA and AIFA in May 2020, the number of test requests increased dramatically until the last year of observation (2022).

4 Discussion

Awareness of the clinical value of *DPYD* testing to limit the risk of severe toxicity to fluoropyrimidines has notably increased over the past decade (Deenen et al., 2011; Meulendijks et al., 2015; Lunenburg et al., 2016; Dalle Fratte et al., 2018). We report here

the experience of a tertiary-level hospital in Italy (National Cancer Institute, CRO-Aviano) with the implementation of *DPYD* genetic polymorphism testing in patients since 2012.

Overall, as with other PGx testing, the adoption of *DPYD* testing in hospitals has been hampered by several previously discussed barriers, such as the need for common national and international pharmacogenetic guidelines, reliable genotyping technology with acceptable turnaround time, and IT technologies suitable for managing genetic data as part of standard clinical workflow (Swen et al., 2011; Amstutz et al., 2018; Martens et al., 2019; Lunenburg et al., 2020; Begré et al., 2022).

Prescribers awareness of the clinical relevance of the tests is considered another relevant barrier to upfront *DPYD* testing in the clinical practice (Formea et al., 2013; Haga et al., 2015; Just et al., 2017, 2019; Giri et al., 2018). The herein reported data show that over the years, not only has the absolute number of tests prescribed increased, but so has the trend from a post-toxicity to a pre-treatment approach, attesting the increasing awareness among oncologists of the importance of adverse drug reactions from *DPYD* genotyping. This could be also related to the active involvement of oncologists in prospective clinical trials such as the U-PGx project (Swen et al., 2023).

The management of genetic data in a clinical context could be another barrier to straightforward implementation of PGx testing in clinical practice (Khelifi et al., 2017). Our diagnostic reporting service has evolved from a paper report delivered only to the prescribing physician, to an electronic report that is included in the patient's health repository and available to any physician with access to the patient's health data (Roncato et al., 2019; Qin et al., 2022). However, we recognize that a clinical decision support system in which the patient's genetic data interact with the medication prescribing system would be the best way to facilitate the integration of genotyping results into the clinical workflow. With this in mind, the CDSS prototype FARMAPRICE was developed with the aim of integrating genetic data into the digital medical record of patients from the CRO-Aviano (Roncato et al., 2019), although no results on clinician acceptance of the tool are currently available. Another approach, within the U-PGx project, was the introduction of the Safety Code Card, a wearable CDSS provided in the patient's hand. However, the latter was hardly adopted by Italian patients in the project, probably due to the high average age of cancer patients, which may affect the ability to use the technologies, or, more simply, to the lack of new drugs prescription given the high mortality rate of the disease (Blagec et al., 2022).

Over the reference time, the number of *DPYD* variants analyzed and the laboratory methods have also changed according to the continuous evolution of the scientific literature and the pharmacogenetic guidelines (Swen et al., 2011; Amstutz et al., 2018; Lunenburg et al., 2020; Abdullah-Koolmees et al., 2021). In the most recent years several European countries developed their own *DPYD* testing panels (Martens et al., 2019; Wörmann et al., 2020; Begré et al., 2022) adding in some cases specific *DPYD* variants in addition to the four variants panel (García-Alfonso et al., 2022). In Italy, a joint committee promoted by SIF-AIOM has developed specific Italian PGx guidelines for *DPYD* testing since 2015, and an updated version was made available in 2019 (SIF-AIOM, 2015; Gori et al., 2019). The *DPYD* pretreatment panel recommended in the SIF-AIOM guidelines is in line with the recommendations of the CPIC and DPWG international consortia. In Italy, an additional *DPYD* variant (*DPYD**6) is recommended for testing in case of severe toxicity, based on the results of some pharmacogenetic association studies reporting a higher risk of toxicity in carriers of this polymorphism (Boige et al., 2016; Ruzzo et al., 2017; Henricks et al., 2018).

The Italian guidelines do not include recommendations for DPD phenotyping by assessing residual DPD enzyme activity from peripheral blood by analysis of uracil (U) and dihydrouracil (UH_2) metabolite plasma concentrations (Van Kuilenburg et al., 1999; Pallet et al., 2020; Ockeloen et al., 2021). Although phenotyping by UH_2/U in peripheral blood mononuclear cells is a direct measure of DPD activity and could reveal a greater number of patients at risk for toxicity, regardless of genetic profile, its application is hampered by several technical limitations. The lack of standardization in the timing of blood collection and processing protocols may influence results, and makes it difficult to directly correlate this ratio with the enzyme activity (de With et al., 2022). Although this is a valuable approach whose effectiveness is demonstrated by its acceptance in other countries such as France (Laures et al., 2022), a DPD phenotyping service is poorly provided by Italian public laboratories.

The lack of clear reimbursement strategies remains a critical barrier to the implementation of pharmacogenetic testing in practice worldwide, in some cases limiting the use of *DPYD* testing to funded projects only (Faulkner et al., 2012; Luzum et al., 2017). Many health economic issues are autonomously managed by different Italian regions. In our case, this led to inhomogeneity in the possibility of having the test reimbursed on the Italian territory. In the Friuli Venezia Giulia region, where our center is based, the pharmacogenetic test has been reimbursed since 2014. This was the first event that improved the uptake of the test by clinicians, as the number of patients referred to the *DPYD* genotyping service doubled between 2013 and 2014. The *DPYD* analysis service at the National Cancer Institute CRO-Aviano was made available to patients referred to the hospital as well as to patients from outside hospital at the regional and national level and become a benchmark for several national centers.

However, the crucial step that led to the inclusion of *DPYD* testing in the clinical practice of our center was the introduction of specific recommendations for DPD testing before fluoropyrimidines prescription by the European (EMA) and Italian (AIFA) regulatory authorities (EMA, 2020; aifa.gov, 2020). Since 2020, pre-treatment *DPYD* testing has been reimbursed in Italy. As our results show, the number of patients tested for *DPYD* before treatment almost doubled between 2020 and 2021 to reach a stable plateau of almost 400 inpatients per year, which is more than 90% of the average number of patients prescribed a fluoropyrimidine in our center in 2022.

Although our results are based on a unique observation point in Italy, where early adoption of testing was driven by specific local health policies and participation in important international pharmacogenetic projects, we observed that similar trends were reported in other European contexts. In recent years, some examples of the introduction of *DPYD* testing into the clinical practice with the support of local health authorities have been reported (Martens

et al., 2019; Wörmann et al., 2020; Begré et al., 2022; García-Alfonso et al., 2022). Recently, a large survey was conducted in several European countries, including Italy, providing an overview of the status of DPD testing implementation in Europe and how this was affected by the publication of the EMA recommendation in 2020. As in the herein presented results, the EMA recommendation was the key event affecting the number of test prescriptions and the revision of national reimbursement guidelines, stimulating the publication of national guidelines in most European countries (de With et al., 2023).

5 Conclusion

DPYD testing is widely recognized as an important strategy to increase fluoropyrimidines treatment safety, however, its implementation in clinical practice is still struggling to become part of routine testing in some parts of the world (Baker et al., 2023). The example of the implementation pathway in our center in Italy shows once again that the success of this process depends on several factors, including disclosure of the value of *DPYD* testing among stakeholders, standardization of laboratory workflows, and adoption of straightforward IT technology. However, the final and critical step for implementing the test into routine practice is the availability of a clear regulatory recommendation by drug regulatory authorities and the establishment of a reimbursement policy.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

Conceptualization: AB, EC, and GT; Data collection: AB, LS, EP, MD'A, and FS; Data analysis; AB, ED, RR, and EC; Writing Original Draft: AB and EC; Manuscript Review and Final Approval: All authors. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.All authors contributed to the article and approved the submitted version

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Pharmacogenomics in practice: a review and implementation guide

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Considerable efforts have been exerted to implement Pharmacogenomics (PGx), the study of interindividual variations in DNA sequence related to drug response, into routine clinical practice. In this article, we first briefly describe PGx and its role in improving treatment outcomes. We then propose an approach to initiate clinical PGx in the hospital setting. One should first evaluate the available PGx evidence, review the most relevant drugs, and narrow down to the most actionable drug-gene pairs and related variant alleles. This is done based on data curated and evaluated by experts such as the pharmacogenomics knowledge implementation (PharmGKB) and the Clinical Pharmacogenetics Implementation Consortium (CPIC), as well as drug regulatory authorities such as the US Food and Drug Administration (FDA) and European Medicinal Agency (EMA). The next step is to differentiate reactive point of care from preemptive testing and decide on the genotyping strategy being a candidate or panel testing, each of which has its pros and cons, then work out the best way to interpret and report PGx test results with the option of integration into electronic health records and clinical decision support systems. After test authorization or testing requirements by the government or drug regulators, putting the plan into action involves several stakeholders, with the hospital leadership supporting the process and communicating with payers, the pharmacy and therapeutics committee leading the process in collaboration with the hospital laboratory and information technology department, and healthcare providers (HCPs) ordering the test, understanding the results, making the appropriate therapeutic decisions, and explaining them to the patient. We conclude by recommending some strategies to further advance the implementation of PGx in practice, such as the need to educate HCPs and patients, and to push for more tests' reimbursement. We also guide the reader to available PGx resources and examples of PGx implementation programs and initiatives.

KEYWORDS

guidelines, implementation, pharmacogenomics, practice, pharmacogenetics

1 Introduction

Interindividual variability in drug response is driven by several extrinsic and intrinsic factors, with genetic variations being increasingly recognized among these factors that lead to changes in the activity or availability of drug metabolizing enzymes (DMEs), receptors, channels, and other proteins involved in drug pharmacokinetics (PK) and pharmacodynamics (PD) (Thummel and Lin, 2014). Consequently, the term

Pharmacogenomics (PGx), the study of interindividual variations in DNA sequence related to drug efficacy and toxicity, was coined. In this sense, PGx has become an effective tool to fulfill the promise of personalized medicine, while allowing patients to be treated based on their genetic makeup (Mitri et al., 2010; Bartlett et al., 2012).

Despite all emerging evidence and efforts enabling PGx, its clinical implementation has been suboptimal worldwide, especially in the developing world (Abou Diwan et al., 2019; Zgheib et al., 2020; El Shamieh and Zgheib, 2022). For instance, and in addition to global challenges such as the perceived lack of clinical utility and worries of disrupting the usual clinical pathways, other barriers may be attributed to local circumstances such as absence of national regulations for PGx testing, suboptimal infrastructure for the PGx integration into healthcare providers' (HCP) workflow, lagging insurance plans for coverage of PGx testing, and lack of resources including national PGx data, guidelines, and necessary funds (Caraballo et al., 2017; Rigter et al., 2020; Pirmohamed, 2023).

Considerable efforts have been exerted to implement PGx into routine clinical practice. These efforts relied upon studies providing robust evidence for the benefit of PGx-guided therapeutic strategies. For instance, it has been reported that approximately 91%–99% of patients have at least one genotype that is associated with PGx actionable drugs, and that these drugs constitute up to 18% of all prescribed medications (Krebs and Milani, 2019). Moreover, a recently published study from the European Ubiquitous Pharmacogenomics (U-PGx) clinical implementation project showed that patients with PGx actionable test results, when treated according to Royal Dutch Association for the Advancement of Pharmacy - Pharmacogenetics Working Group (DPWG) recommendations, resulted in a lower percentage (21%) of clinically relevant adverse drug reactions (ADRs) compared to the control group (27.7%) that received standard treatment, though this difference was also seen for patients in the case group receiving nonactionable drugs (Swen et al., 2023). Further evaluations can be pursued in this study to address the influence of several genes, specific adverse reactions related to individual drugs, and phenoconversion caused by polypharmacy (Penas, 2023). In addition, it has been shown that ADRs and hospitalization resulting from drug toxicity can be better controlled by applying PGx. Cost savings from PGx-guided therapy can reach up to 3962 USD per patient per year even when test costs are considered (Luzum et al., 2021). More specifically, a systematic review that evaluated PGx-guided treatment of antidepressants and antipsychotic medications showed that 50% and 39% of the included studies revealed cost-effectiveness and cost-saving of PGx testing, respectively (Karamperis et al., 2021).

Considering the extensive evidence on the benefits of PGx and the availability of a myriad of resources enabling its clinical implementation, herein we propose an approach for initiating clinical PGx in the hospital setting, while acknowledging that implementation depends on local circumstances such as resources available, differences in insurance plans, and peculiarities of the health service's organization, etc .,. . . To begin with, we introduce the stakeholders engaged in the implementation, evaluation, and improvement of the program. Next, we propose steps to be followed for developing and applying PGx in hospital clinical practice. We then discuss strategies to address the PGx awareness and training needs of HCPs and patients, and elaborate on the necessity of test reimbursement and how it can be enhanced. We also guide the reader to available PGx resources, and examples of PGx implementation programs and initiatives.

2 Stakeholders engaged in the clinical pgx design and implementation process

At least eight main stakeholders are involved in the PGx design and implementation process in the hospital setting (Figure 1; Box 1). These include drug regulators authorizing or requiring specific PGx tests, hospital leadership supporting the process and communicating

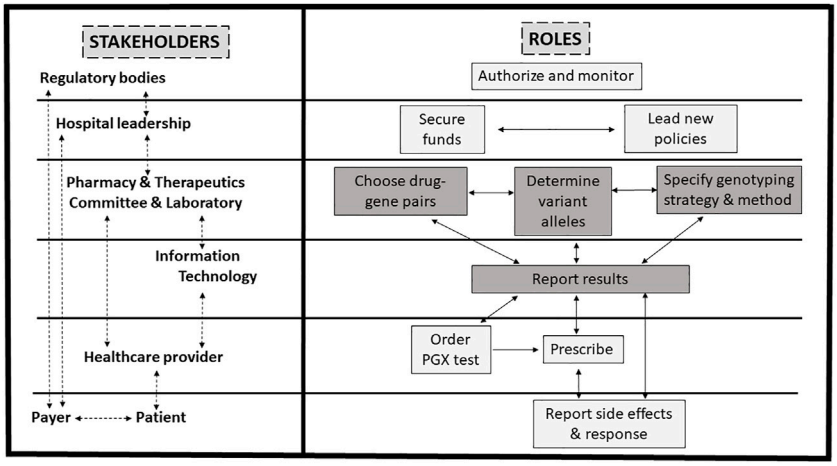

FIGURE 1
Proposed framework for pharmacogenomics (PGx) implementation in practice. PGx implementation in practice involves several stakeholders. See Box 1 for details. Briefly, after test authorization or requirements for testing by drug regulators, the hospital leadership supports the process and communicates with payers, while the pharmacy and therapeutics committee leads the process in collaboration with the hospital laboratory and information technology department. Healthcare providers order the test, make the appropriate therapeutic decisions, and explain them to the patient whom reports to the healthcare provider and payer. Steps for the design of the clinical PGx are highlighted in grey and detailed in Figure 2.

BOX 1 List and role of main stakeholders involved in the pharmacogenomics (PGx) implementation process in a hospital setting

Stakeholder	Role
Regulatory bodies	Authorize or require specific PGx tests.
	Provide guidelines and/or drug labeling.
	Monitor implementation.
	Communicate with hospital leadership and payers.
Hospital leadership	Secure funds and infrastructure.
	Lead the process and identify early adopters of change.
	Ensure compliance with ethical, legal and social issues.
	Monitor and evaluate the program's impact.
	Contribute to and lead new policies.
	Communicate with regulatory bodies, payers, and pharmacy and therapeutics committee.
Pharmacy & Therapeutics Committee	Evaluate the available evidence in consultation with PGx consortia and networks.
	Review the most relevant drugs.
	Narrow down to the most actionable drug-gene pairs with related variant alleles.
	Monitor and evaluate the program for improvement.
	Communicate with hospital leadership, laboratory, information technology and healthcare providers.
Laboratory	Perform genotyping.
	Apply reactive point of care or preemptive testing.
	Choose candidate vs. panel testing.
	Communicate with pharmacy and therapeutics committee, information technology and healthcare providers.
Information Technology	Report and integrate results into electronic health records.
	Design clinical decision support systems.
	Communicate with pharmacy and therapeutics committee, laboratory, and healthcare providers.
Healthcare provider	Order the test.
	Understand and interpret the test results.
	Make therapeutic decisions.
	Educate the community.
	Communicate with patients, pharmacy and therapeutics committee, laboratory, and information technology.
Patient	Provide feedback on drug outcome
	Communicate with payers and healthcare providers.
Payer	Reimburse (or not) the test partially or fully
	Communicate with regulatory bodies, hospital leadership and patients.

with payers, pharmacy and therapeutics (P&T) committee leading the process in collaboration with the hospital, molecular laboratory and information technology (IT), and HCPs ordering the test, understanding the results, making the appropriate therapeutic decisions, and explaining them to the patients.

2.1 Regulatory bodies

The FDA and EMA regulatory bodies in the US and Europe, respectively, are responsible for regulating the addition of PGx information and assessing the level of PGx labeling, be it required, recommended, actionable or informative (Ehmann et al., 2014; Mehta et al., 2020). Their recommendations are available through PharmGKB website, where HCPs can access all corresponding prescribing information and recommendations. In addition, guidelines on PGx data usage in drug development and labeling were established by the FDA and EMA. According to the FDA (United States Food and Drug Administration, 2005), the sponser may choose to submit with an investigational or marketing application PGx data that have not yet reached the status of a valid biomarker to, for example, correlate specific toxicities with genetic

data, or inform the design of clinical trials. However, when the PGx data are known to affect safety in animals or efficacy or safety in humans, it is recommended that such data are submitted with the application. As for labeling, the PGx data may be included in an informational or actionable manner. Concerning the EMA (European Medicines Agency, 2010), and in the case of co-development of PGx biomarkers or assays, guidelines are put in place while reflecting on key scientific principles that need to be met to ensure compliance with good laboratory standards resulting in optimal reliability of the PGx assay.

Moreover, in the USA, the National Human Genome Research Institute encourages research conducted on health benefits and cost-effectiveness of genetic testing to promote genomic medicine (Vozikis et al., 2016). It also supports payers to enable reimbursement of genetic tests. On the other hand, in Europe, there are national regulatory bodies for each country, such as the Gemeinsamer Bundesausschuss in Germany, the Medicines and Healthcare Regulatory Agency in the United Kingdom, and La Haute Autorité de Santé in France (Vozikis et al., 2016). Depending on specific national regulations, they are responsible for authorizing, marketing, and/or monitoring the quality and safety of medicinal products. Their role is tuned by the government to ensure cooperation between various stakeholders -regulatory authorities, medical device manufacturers, payer organizations, academic/research institutes, wholesalers, laboratories, pharmaceutical companies, and HCPs-to ensure availability and affordability of medical supplies including genetic tests. Tests that were shown to improve healthcare were proposed to be included in a "positive medical device" list to enforce its use and reimbursement by public and private insurance companies (Vozikis et al., 2016).

2.2 Hospital leadership

The leadership group is the initial sponsor of the program. It is responsible for securing funds and infrastructure, ensuring compliance with ethical legal social issues (ELSI), and monitoring and evaluating the program's impact. The leadership is fully engaged in the whole process and should be sensitive to the hospital culture and climate, including the readiness for change. It should identify early adopters of change or implementation champions (Tuteja et al., 2022). It may also present evidence to national officials to suggest amending regulations in favor of promoting the practice of personalized medicine, and developing reimbursement policies and educational programs (Hartzler et al., 2013; Cicali et al., 2022).

2.3 Developers: pharmacy and therapeutics committee, laboratory and information technology

Then comes the role of the program developers being the P&T committee in collaboration with the hospital's laboratory and IT department. The P&T is a multidisciplinary committee that is responsible for all matters related to the use of medications in the institution, including the development and maintenance of the hospital formulary. For the sake of the proposed PGx program, we suggest the P&T committee, while in constant communication with expert PGx consortia and networks, to be responsible for evaluating the available PGx evidence, reviewing the most relevant drugs, and narrowing down to the most actionable drug-gene pairs with related variant alleles to be tested. P&T members also discuss and decide with laboratory experts whether to apply reactive point of care or preemptive testing and on the genotyping strategy being a candidate or panel testing. The P&T committee also collaborates with IT to find the best way to report and interpret PGx test results with the option of integration into electronic health records (EHRs) coupled with clinical decision support (CDS) systems. It follows the program's progress for improvement (Hartzler et al., 2013; Cicali et al., 2022).

2.4 Users: healthcare providers and patients

After that comes the role of the program users, being HCPs and patients. HCPs order the PGx test paired with the drug they plan to prescribe for a specific therapeutic need, interpret test results, communicate with patients, prescribe the personalized dose, or choose an alternative medication as applicable. HCPs also have an essential role in educating the community and patients on PGx and how it can impact their treatment. They can help monitor the general attitude toward PGx implementation and propose strategies to increase PGx awareness. These include educational workshops and conferences, TV and social media talks, and billboard and brochure advertisements that introduce the program to the public. HCPs also must provide feedback to the P&T, the molecular laboratory, and IT personnel regarding the process of including CDS in order to enhance the efficiency and efficacy of the implemented system (Hartzler et al., 2013; Cicali et al., 2022). Patients' attitudes towards the PGx testing should also be taken into consideration. They should be informed about regulations that protect them from genetic discrimination by insurance companies and employers. They also should be informed regarding reimbursement policies and whether testing is entirely, partially, or not covered (Hartzler et al., 2013; Cicali et al., 2022).

2.5 Payers

Finally comes the role of payers, which may be public or private health insurance plans, research grants, laboratory reimbursement plans, out-of-pocket, or others. Although many potential payers are still reluctant to reimburse the PGx implementation or test, the growing evidence on the clinical utility of PGx testing is pushing toward fulfilling the right of patients to receive individualized treatment and to be protected by public policies and regulations that are integrated into national public or private health plans (Tuteja et al., 2022). Establishing or updating well-defined regulations will ultimately force insurance companies to revise their coverage plans to enable PGx testing. A success story is the experience of genotyping for *DPYD* variant alleles upon fluoropyrimidines prescribing whereby the resulting clinical and economic benefits led to securing governmental financial support in Ontario, with further evidence of cost-effectiveness probably leading to expansion of the experience to other medical institutions (Brooks et al., 2022; Medwid and Kim, 2022; Varughese et al., 2022).

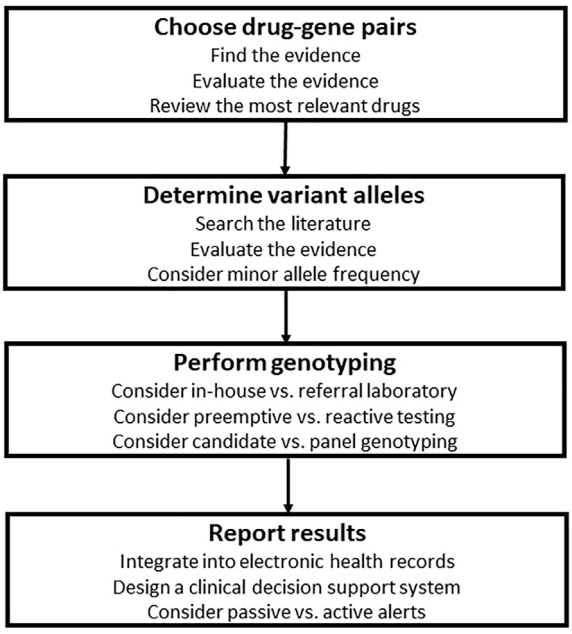

FIGURE 2
Steps for the design of clinical pharmacogenomics (PGx) in the hospital setting. The first step is to evaluate the available PGx evidence, review the most relevant drugs, and narrow down to the most actionable drug-gene pairs, then is to choose variant alleles based on population specific minor allele frequencies and data curated and evaluated by experts and drug regulatory authorities. Next is to decide with the molecular laboratory on the genotyping strategies and methods. Then is to work out with the information technology team the best way to interpret and report PGx test results, ideally into patients' electronic health records if available, and with clinical decision support systems if feasible.

Moreover, the EMA recommended testing for DPYD, but concerns regarding the economic benefit and cost-effectiveness of testing resulted in slow adoption of the recommendation. Thus, studies were initiated in some countries to address cost-effectiveness and potential for improvement of quality of life as a result of DPYD genotyping prior to fluoropyrimidine-based chemotherapy (Deenen et al., 2016; Brooks et al., 2022). One study showed that DPYD screening is a cost-effective strategy and improves survival by 0.0038 quality adjusted life years (Brooks et al., 2022). Another study showed that genotype-guided dosing reduces grade 3 and above toxicity from 73% to 28%, drug-induced death from 10% to 0%, and average treatment cost per patient (Deenen et al., 2016). The EMA recommendation resulted in a requirement for DPYD genotyping in the UK (Tsiachristas et al., 2022). Also, guidelines for DPYD testing have been issued in other European countries such as The Netherlands, Italy, Germany and France (Tsiachristas et al., 2022) with a mandatory character.

3 Design of clinical pgx in the hospital setting

As shown in Figures 1, 2, designing a clinical PGx program in the hospital setting entails several steps. The P&T committee should evaluate the available PGx evidence, review the most relevant drugs, and narrow down to the most actionable drug-gene pairs and related variant alleles based on data curated and evaluated by experts and drug regulatory authorities. The next step is to decide with the molecular laboratory on the genotyping strategies and methods. Then is to work out with the IT team the best way to interpret and report PGx test results, ideally into patients' EHRs if available, and with CDS systems if feasible.

3.1 Choosing the top drug-gene pairs

In order to choose the top drug-gene pairs to be initially implemented, the P&T committee should first consult external expert sources such as PGx consortia and networks to find and evaluate the most substantial evidence for PGx testing. Then, the list of drugs and related genes can be narrowed down based on the reviewed evidence.

3.1.1 Finding the evidence

The implementation of PGx programs requires high-quality and consistent evidence that can be translated into regulations and guidelines (Luzum et al., 2021). These can be compiled from available PGx resources such as consortia, networks, societies, and regulatory agencies.

Several consortia and networks, some of which are listed and described in Table 1, were launched in the attempt to increase awareness, facilitate adoption, and provide the guidance necessary for integration of PGx programs into clinical practice. The Pharmacogenomics Global Research Network (PGRN) (Pharmacogenomics Research Network PGRN, 1998) is one of the first professional communities to work on PGx implementation. It has been heading several projects to include the recruitment and genotyping of people as part of a research protocol for the evaluation of the utility of the PGx endeavor on drug response. In addition, and as part of the Electronic Medical Records and Genomics Network (e-MERGE) (Electronic Medical Records and Genomics Network e-MERGE, 2007), the PGRN has been working on and proposing ways to upgrade EHR systems to be compatible with genetic results storage, as well as designing CDS for drug-gene pairs to guide HCPs in test ordering, interpretation, and drug prescription. Similarly, the Implementing Genomics in Practice (IGNITE) (Implementing Genomics in Practice IGNITE, 2013) network provides guidance for genomic implementation in healthcare, and provides a guiding toolbox for clinicians. Moving forward, professional PGx communities and programs progressed to provide improved PGx implementation models, clinical utility evidence, and comprehensive resources. All these efforts produced a set of valuable databases and tools that allow getting information on the drug and genes affecting its response, such as with the Pharmacogenomics Knowledge Base (PharmGKB) (Pharmacogenomics Knowledge Base PharmGKB, 2001; Whirl-Carrillo et al., 2012; Whirl-Carrillo et al., 2021), the genes, variants, frequencies and their phenotype with the Pharmacogene Variation Consortium (PharmVar) (Pharmacogene Variation Consortium PharmVar, 2000; Gaedigk et al., 2018; Gaedigk et al., 2020; Gaedigk et al., 2021), recommendations on what genetic variants should be tested to get interpretable results by the Association of Molecular

TABLE 1 Few programs and resources for the implementation of Pharmacogenomics (PGx).

Program or resource with website link	Description
AMP: Association of Molecular Pathology https://www.amp.org/	➢ It is an international non-profit scientific society that aims to enhance the science and clinical practice of molecular and genomic laboratories
	➢ It provides guidelines and global expertise in the field of molecular pathology
	➢ It also provides recommendations for the choice of genetic variants that ought to be tested
CPIC: Clinical Pharmacogenetics Implementation Consortium https://cpicpgx.org/	➢ It is an international consortium of volunteers and staff that aim to facilitate the use of PGx tests in clinical care
	➢ It creates, curates, and posts freely available, peer-reviewed, evidence-based, updatable, and detailed gene/drug clinical practice guidelines
	➢ All guidelines are published in the Clinical Pharmacology and Therapeutics journal
e-MERGE: Electronic Medical Records and Genomics Network https://emerge-network.org/	➢ It is a US network of academic medical centers that integrate genomic data with EHR
	➢ It aims to sequence and clinically implement relevant genotypes into healthcare through EHR and CDS incorporation. It also aims to discover and assign phenotypes of rare and presumably clinically relevant variants
	➢ It provides resources and tools (informatics, education) including EHR and CDS infrastructure to assist in the implementation of PGx into practice. CDS-KB (Clinical Decision Support Knowledgebase) (https://cdskb.org/) is one of the tools that is supported by *e-MERGE* in collaboration with *IGNITE* shown below
IGNITE: Implementing Genomics in Practice https://gmkb.org/ignite-gdp/	➢ It is a US network that supports genomic implementation in healthcare setting
	➢ It aims to develop, use, and evaluate new strategies and clinical models for implementing individuals' genomic information into clinical practice
	➢ It provides a Toolbox for clinicians that consists of a collection of genomic practice models related to disease diagnosis, pharmacogenomics and risk assessment. And for researchers, it provides guides and educational material on data collection, laboratory testing, research and training development tools. It has also developed a map for reimbursement of PGx tests
PGRN: Pharmacogenomics Global Research Network https://www.pgrn.org/what-is-pgrn.html	➢ It is a community driven international network that includes academic institutions, diagnostic laboratories, biotechnology, pharmaceutical industry, and clinical practitioners
	➢ It aims to guide and lead precision medicine for actionable variants, and to establish a worldwide collaboration of PGx researchers with a focus on supporting PGx in developing countries
	➢ It provides its members with links to implementation resources, algorithms for PGx-based dosing, PGx competencies for teachers, Research-in-Progress Seminar series (RIPS), and patient education
PharmCAT: Pharmacogenomics Clinical Annotation Tool https://pharmcat.org/	➢ It is a software tool that can extract CPIC PGx variants and represent them with the suitable star allele haplotype/diplotype
	➢ It provides interpretation, and generates a report for the variant alleles
PharmGKB: Pharmacogenomics Knowledge Base https://www.pharmgkb.org/	➢ It is a publicly available resource that is responsible for the integration and dissemination of information related to genomic variation and drug response
	➢ It aims to help healthcare providers and researchers find information about genetic polymorphisms and their effect on drugs' efficacy and safety
	➢ The website includes information and links to curated pathways, Very Important Pharmacogenes (VIP), PGx prescribing information, drug label PGx annotations, as well as PGx variant and clinical annotations based on updated evidence-based criteria
PharmVar: Pharmacogene Variation Consortium https://www.pharmvar.org/	➢ It is a central repository for PGx haplotypes and allelic variants with a focus on drug metabolizing enzymes
	➢ It aims to facilitate basic and clinical research and the interpretation of PGx tests' results
	➢ It also provides a unifying designation system (nomenclature) for the global PGx community

EHR: electronic health records; CDS: clinical decision support.

Pathology (AMP) (Association of Molecular Pathology AMP, 1995), templates for creating genotyping result reports by the Pharmacogenomics Clinical Annotation Tool (PharmCat) (Sangkuhl et al., 2020; Pharmacogenomics Clinical Annotation Tool PharmCat, 2022), and recommendations on what to do when a PGx drug is prescribed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (Clinical pharmacogenetics implemetation Consortium CPIC, 2009).

In addition to the above-described CPIC, few other professional societies have established guidelines for PGx practice (Pharmacogenomics Knowledge Base PharmGKB, 2017), including the DPWG (The Dutch Pharmacogenetics Working Group DPWG, 2005), the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) (Canadian Pharmacogenomics Network for Drug Safety CPNDS, 2004) and the French National Network of Pharmacogenetics (RNPGx) (Picard et al., 2017), among others. In addition, drug regulatory agencies (Pharmacogenomics Knowledge Base PharmGKB, 2017) including the EMA (European Medicinal Agency EMA, 1995) and the US FDA, have incorporated PGx information and prescribing tags in the approved drug labels, with the FDA allocating a specific and regularly updated link to all approved drugs with PGx label annotations (Mehta et al., 2020; United States Food and Drug Administration, 2022a).

3.1.2 Evaluating the evidence

The P&T may choose to build on guidelines or regulations established in one's country if available. For many countries, however, such regulations are not available, institutions would hence have to compare and contrast various resources and choose what is most applicable to their local context. The Office of Public Health Genomics at the US Center for Disease Control and Prevention (CDC) proposes a model for the evaluation and integration of genomic tests based on four components, A, C, C, and E, with A being analytical validity that addresses the accuracy and reliability of genetic testing, C being clinical validity that looks at the accuracy and reliability by which the test predicts the associated drug outcome, C being the clinical utility as the risks and benefits resulting from introducing genetic tests into clinical practice on the community, and E for ELSI being the associated ethical and regulatory policies (Centers for Disease Control and Prevention, 2000).

Similar frameworks have been applied by the FDA and EMA regulatory authorities and the PharmGKB to come up with evidence-based annotation levels, and the CPIC for their implementation guidelines. All provide recommendations on whether to use a drug, adjust the dose or switch to an alternative based on rigorously evaluated evidence. The PharmGKB curates and analyzes available studies to provide annotations for drugs and gene variants, while assigning a level of evidence based on an elaborate scoring system that depends on two main factors. First, the variant annotation score is calculated by a stepwise process that considers all aspects of the evaluated studies including phenotype category, *p*-value, cohort and effect size, study type and the presence of a significant association. Second is the presence of a clinical guideline and or a drug label. Level of evidence for drug-gene variants ranges from 1 to 4, with 1A being supported by solid and non-conflicting data, while pairs assigned a level of evidence of 4 lack supporting data (Whirl-Carrillo et al., 2012; Whirl-Carrillo et al., 2021). As for CPIC, A, B, C, and D levels are designated such that A and B imply that evidence favors changing the drug prescription to a genetically safer one. In contrast, C and D imply that evidence did not reach a level to suggest a genetically-based prescription. CPIC also applies a framework to rank its recommendations as strong, moderate, or optional based on supporting studies such as randomized clinical trials (RCTs) and *in vivo* PK/PD studies (Caudle et al., 2016).

3.1.3 Narrowing down to a list of top drug-gene pairs

We propose that institutions that do not have country-specific guidelines or regulations compile all available PGx data from the PharmGKB, CPIC, FDA, and EMA. See Supplementary Table S1 as an example. We tabulated all drugs that are either listed in the PharmGKB's Drug Label Annotations table under FDA or the Clinical Guideline Annotations table under CPIC. We also added the PharmGKB level of evidence for clinical annotations and all CPIC recommendations, and FDA and EMA PGx levels and drug labels when available. Of note that some discordance can be noted among the various resources despite being derived from the same evidence base (Koutsilieri et al., 2020; Shekhani et al., 2020; United States Food and Drug Administration, 2022b; Pirmohamed, 2023). For instance, many of the drugs labeled as "testing required" by the FDA are not mentioned in EMA labels. Moreover, many drug-gene associations listed by the FDA are neither listed by EMA nor by CPIC (See Supplementary Table S1). More specifically for clopidogrel prescription, for example, CPIC (Scott et al., 2013) and FDA (United States Food and Drug Administration, 1997) recommend the use -or consideration of the use-of alternative drugs in CYP2C19 poor metabolizers. At the same time, the EMA label (European Medicines Agency, 1998) does not make such a specific recommendation. Hence the role of the P&T committee to assess these inconsistencies and to make an informed decision on what model to follow and what drugs to include.

Two methods can be applied concomitantly or independently to narrow down the list of drug-gene pairs to be initially implemented. First, one can evaluate and choose the most frequently prescribed drugs in one's setting. For example, a program initiated in Africa should include drugs like chloroquine, HIV-protease inhibitors, and isoniazid used to treat malaria, HIV, and tuberculosis, respectively (Greenwood, 2004; Marais et al., 2013; Dandara et al., 2019), while recognizing that non-communicable diseases are also an important cause of morbidity and mortality in developing countries (Grant and De Cock, 1998; Kennedy et al., 2007; Dean et al., 2020). If such data are unavailable, one can refer to the World Health Organization (WHO) list of essential drugs (World Health Organization WHO, 2021). The second method is to review available literature in other institutions or countries on the most commonly prescribed drugs supported by solid evidence of clinical utility for PGx. Several studies were conducted on multiple populations (Schildcrout et al., 2012; Samwald et al., 2016; Caraballo et al., 2017; Chanfreau-Coffinier et al., 2019; Hicks et al., 2021). Samwald et al. (2016) classified the most frequently used PGx drugs in the USA within different age groups based on a model whereby PGx drug exposure data were collected from insurance databases, the drugs that had CPIC or DWPG guidelines were then highlighted, followed by the selection of the most frequently used drugs, while considering different ethnic

TABLE 2 Proposed drug-gene pairs for the clinical implementation of pharmacogenomics (PGx).

Indications	Drugs			Genes	PHARMGKB[b] https://www.pharmgkb.org/	CPIC[c] https://cpicPGx.org/		FDA[d] https://www.fda.gov/		EMA[e] https://www.ema.europa.eu/en	
	Classes	Name	On WHO list of essential medicines[a]		Level of evidence of PGx clinical annotation	Recommendation	Drug label annotation Tag	PGx level	Drug label annotation Tag	PGx level	Drug label annotation Tag
Autoimmune Diseases	Antigout	Allopurinol	Yes	HLA-B	1A	Yes	Pediatric	Testing Recommended	Alternate drug, Prescribing info	-	-
Cardiovascular Diseases	Anticoagulant	Warfarin	Yes	CYP2C9, VKORC1	1A	Yes	Pediatric	Actionable PGx	Prescribing Info	-	-
				CYP4F2	1A	Yes	Pediatric	-	-	-	-
	Antiplatelet	Clopidogrel	Yes	CYP2C19	1A	Yes	Pediatric	Actionable PGx	Prescribing Info	Actionable PGx	-
	Statins	Atorvastatin	Yes	SLCO1B1	1A	Yes	Dosing info, Pediatric	Informative PGx	-	-	-
		Rosuvastatin	-	ABCG2, SLCO1B1	1A	Yes	Alternate drug, Dosing info, Pediatric	Actionable PGx	-	-	-
		Simvastatin	Yes	SLCO1B1	1A	Yes	Alternate drug, Dosing info, Pediatric	Informative PGx	-	-	-
Gastrointestinal diseases	Antiemetic	Ondansetron	Yes	CYP2D6	1A	Yes	Pediatric	Informative PGx	-	-	-
	Proton pump inhibitors	Dexlansoprazole	-	CYP2C19	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Lansoprazole	-	CYP2C19	1A	Yes	Pediatric	Informative PGx	-	-	-
		Omeprazole	Yes	CYP2C19	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Pantoprazole	-	CYP2C19	1A	Yes	Pediatric	Actionable PGx	Prescribing info, Pediatric	-	-
Infectious diseases	Antibiotics	Gentamicin	Yes	MT-RNR1	1A	Yes	Alternate drug, Pediatric	-	-	-	-
		Streptomycin	-	MT-RNR1	1A	Yes	Alternate drug, Pediatric	-	-	-	-
		Tobramycin	Yes	MT-RNR1	1A	Yes	Alternate drug, Pediatric	-	-	-	-
	Antifungal	Voriconazole	Yes	CYP2C19	1A	Yes	Pediatric	Actionable PGx	-	Informative PGx	-
	Antivirals	Abacavir	Yes	HLA-B	1A	Yes	Pediatric	Testing Required	Alternate drug, Prescribing info	Testing Required	Alternate drug, Prescribing info
		Atazanavir	Yes	UGT1A1	1A	Yes	Pediatric	-	-	-	-
		Efavirenz	Yes	CYP2B6	1A	Yes	Pediatric	Actionable PGx	-	Actionable PGx	-
Neuropsychiatric diseases	Antiepileptics	Carbamazepine	Yes	HLA-A	1A	Yes	Alternate drug, Pediatric	Actionable PGx	-	-	-
				HLA-B	1A	Yes	Alternate drug, Pediatric	Testing Required	Alternate drug, Prescribing info	-	-
		Oxcarbazepine	-	HLA-B	1A	Yes	Alternate drug	Testing Recommended	Alternate drug, Prescribing info	-	-
		Phenytoin	Yes	CYP2C9, HLA-B	1A	Yes	Pediatric	Actionable PGx	Prescribing info	-	-
	Non-opioid analgesic	Celecoxib	-	CYP2C9	1A	Yes	Pediatric	Actionable PGx	Dosing info, Prescribing info	-	-

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10.3389/fphar.2023.1189976

TABLE 2 (*Continued*) Proposed drug-gene pairs for the clinical implementation of pharmacogenomics (PGx).

Indications	Drugs			Genes	PHARMGKB[b] https://www.pharmgkb.org/	CPIC[c] https://cpicPGx.org/		FDA[d] https://www.fda.gov/		EMA[e] https://www.ema.europa.eu/en	
	Classes	Name	On WHO list of essential medicines[a]		Level of evidence of PGx clinical annotation	Recommendation	Drug label annotation Tag	PGx level	Drug label annotation Tag	PGx level	Drug label annotation Tag
	Opioid analgesics	Codeine	Yes	CYP2D6	1A	Yes	Pediatric	Actionable PGx	Alternate drug, Prescribing info, Pediatric	-	-
		Tramadol	-	CYP2D6	1A	Yes	Pediatric	Actionable PGx	Alternate drug, Prescribing info	-	-
	Antidepressants	Amitriptyline	Yes	CYP2C19	1A	Yes	Pediatric	-	-	-	-
				CYP2D6	-	Yes	Pediatric	Actionable PGx	-	-	-
		Aripiprazole	-	CYP2D6	1A	-	-	Actionable PGx	Dosing info, Prescribing info	Actionable PGx	Dosing info, Prescribing info
		Citalopram	Yes	CYP2C19	1A	Yes	Pediatric	Actionable PGx	Dosing info, Prescribing info	-	-
		Clomipramine	Yes	CYP2C19	1A	Yes	Pediatric	-	-	-	-
				CYP2D6	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Desipramine	-	CYP2D6	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Doxepin	-	CYP2C19, CYP2D6	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Escitalopram	Yes	CYP2C19	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Fluvoxamine	Yes	CYP2D6	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Imipramine	-	CYP2C19	1A	Yes	Pediatric	-	-	-	-
				CYP2D6	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Nortriptyline	-	CYP2D6	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Paroxetine	Yes	CYP2D6	1A	Yes	Pediatric	Informative PGx	-	-	-
		Sertraline	Yes	CYP2C19	1A	Yes	Pediatric	-	-	-	-
		Trimipramine	-	CYP2C19	1A	Yes	Pediatric	-	-	-	-
				CYP2D6	1A	Yes	Pediatric	Actionable PGx	-	-	-
		Venlafaxine	-	CYP2D6	1A	-	-	Actionable PGx	-	-	-
Oncology	Cytotoxic therapy	Capecitabine	Yes	DPYD	1A	Yes	Pediatric	Actionable PGx	Prescribing Info	Testing Recommended	Alternate drug, Prescribing info
		Fluorouracil	Yes	DPYD	1A	Yes	Pediatric	Actionable PGx	Alternate drug, Prescribing info	-	-
		Mercaptopurine	Yes	NUDT15, TPMT	1A	Yes	Pediatric	Testing Recommended	Dosing info, Prescribing info	Actionable PGx	Prescribing Info
		Thioguanine	-	NUDT15, TPMT	3	Yes	Pediatric	Testing Recommended	Dosing info, Prescribing info	-	-

(Continued on following page)

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TABLE 2 (*Continued*) **Proposed drug-gene pairs for the clinical implementation of pharmacogenomics (PGx).**

Indications	Drugs			Genes	PHARMGKB[b] https://www.pharmgkb.org/	CPIC[c] https://cpicpgx.org/		FDA[d] https://www.fda.gov/		EMA[e] https://www.ema.europa.eu/en	
	Classes	Name	On WHO list of essential medicines[a]		Level of evidence of PGx clinical annotation	Recommendation	Drug label annotation Tag	PGx level	Drug label annotation Tag	PGx level	Drug label annotation Tag
	Hormonal therapy	Tamoxifen	Yes	CYP2D6	1A	Yes	-	Actionable PGx	-	-	-
	Immunosuppressants	Azathioprine	Yes	NUDT15, TPMT	1A	Yes	Pediatric	Testing Recommended	Prescribing info	-	-
		Tacrolimus	Yes	CYP3A5	1A	Yes	Pediatric	Informative PGx	-	-	-

[a]https://www.who.int/publications/i/item/WHO-MHP-HPS-EML-2021.02

[b]PharmGKB, clinical annotations levels range from 1-4, with level 1 meeting the highest criteria.

[c]CPIC, provides guidelines that help in implementing pharmacogenomics tests into clinical practice setting.

[d]FDA, approves and monitors drugs and other biological products for ensuring safety and effectiveness in the United States of America.

[e]EMA, approves and monitors drugs and other biological products for ensuring safety and effectiveness within the European union and economic area.

Testing Required: Genetic testing is obligatory before prescribing the drug.

Testing Recommended: Genetic testing should be considered before prescribing the drug.

Actionable PGx: Genetic testing is available and advised due to possible gene-drug relation, but it is not required.

Informative PGx: Genetic testing is not required. Gene variants do not affect drug response, or the effect is not clinically significant.

Dosing info: Tag implies that dose adjustment is required.

Alternate drug: Tag implies that a drug substitute is recommended.

Prescribing info: Tag implies that dose adjustment or drug substitution is either required or suggested, drug should be used with caution, or patients should be monitored for adverse reactions.

Pediatric: Tag implies that a drug contains PGx information for pediatric population.

groups. This analysis showed that opioids (codeine, oxycodone, and please inset here: groups. This analysis showed that opioids (codeine, oxycodone, and tramadol) were primarily used in the younger population, while cardiovascular drugs (simvastatin, clopidogrel, and warfarin) were frequently prescribed for older people (Samwald et al., 2016; Hicks et al., 2021). Similar results appeared from a survey conducted by IGNITE group with 11 healthcare systems, whereby each site provided the available e-prescription records of adults above 18 years for in and outpatient settings (Hicks et al., 2021). A third study conducted at Vanderbilt University Medical Center reached a similar conclusion, adding some oncology drugs to the top 25 PGx most prescribed drugs (Schildcrout et al., 2012). In this study, drugs having FDA PGx prescribing information were considered. Consequently, a list of drugs was suggested by several investigators as ready to be implemented. This list includes but is not limited to: statins, clopidogrel, and warfarin for cardiovascular diseases, codeine and tramadol as opioid analgesics, ondansetron and proton pump inhibitors for gastrointestinal illnesses, fluoropyrimidines, tamoxifen, and thiopurines for cancer, and antidepressants such as the selective serotonin reuptake inhibitors (Weitzel et al., 2019; Rollinson et al., 2020; Medwid and Kim, 2022). Finally, a fourth study evaluated the longitudinal exposure in primary care in the United Kingdom of a list of 63 drugs identified from the PharmGKB database to be associated with 19 pharmacogenes. The authors showed that most of the prescribed PGx drugs were for pain relief, gastrointestinal protection, and psychiatric and cardiovascular conditions, and that more than 95% of these drugs are affected by three pharmacogenes: CYP2C19, CYP2D6, and SLCO1B1 (Kimpton et al., 2019).

Based on these two methods, we narrowed down the list of 499 drugs from Supplementary Table S1 into 59 drugs with related pharmacogenes (Table 2). We limited the list to drugs associated with germline non-somatic PGx tests. As such, we did not include genes for targeted anticancer drugs or immunotherapy. We also checked the Tier 1 Very Important Pharmacogenes (VIP) list from PharmGKB (Pharmacogenomics Knowledge Base PharmGKB, 2020), and kept some drug-gene pairs such as TPMT and NUDT15 for thioguanine despite being non-level 1A per PharmGKB level of evidence. We chose so for the mere fact that both VIP are also associated with more substantial evidence with other drugs such as mercaptopurine and azathioprine. We excluded VIP for drugs not commonly used in the community worldwide such as CFTR, F5, and RYR1 related to cystic fibrosis treatment, thrombopoeitin receptor agonists, and anesthetic drugs, respectively. We also did not include NAT2 for isoniazid as the drug-gene pair has so far not been addressed by the CPIC.

3.2 Choosing the variants to be tested

After determining the drug-gene pairs, the P&T committee should specify the variants that should be tested in coordination with Laboratory experts and personnel. We describe in Table 3 a list of variants associated with the genes proposed in Table 2. This list is non-exhaustive and laid out for illustrative purposes only. It is based on the most commonly proposed variants and genes in the literature (Samwald et al., 2016; van der Wouden et al., 2017; Chanfreau-

Coffinier et al., 2019; van der Wouden et al., 2019; Rollinson et al., 2020; Medwid and Kim, 2022) and supported with high quality evidence, clinical guidelines and/or drug labels with genetic information. We also chose variants that are relatively common in Africans, Asians or Europeans. Each genetic variant has a unique identifier (rsID) as per the NCBI dbSNP database (National Center for Biotechnology Information NCBI, 1999). The *allele nomenclature and genotype-phenotype relation can be extracted from the PharmGKB and/or PharmVar resources.

The most crucial consideration for the choice of the target variants is the Minor Allele Frequency (MAF) of the local population or ethnicities (Van Driest et al., 2014). For instance, while in East Asians, HLA-B*15:02 allele is relatively common, and its PGx testing for carbamazepine is required to prevent Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), the same test is not required in other populations where the allele is quite rare (United States Food and Drug Administration, 2009). Another example is mercaptopurine, whereby only TPMT genotyping is recommended in Caucasians, while both TPMT and NUDT15 genotyping should be performed for East Asians who may carry some common actionable NUDT15 variants (Yang et al., 2015). A third example comprises CYP2C9 genotyping test before prescribing warfarin to African Americans, whereby the test should include CYP2C9 *2, *3, *5, *6, *8, and *11 *versus* only *2 and *3 for Europeans (Johnson et al., 2017). Another important consideration to be kept in mind is how well the variants reflect the phenotype of the enzyme activity. For this purpose, the AMP guides on the basic variants that should be tested together to allow accurate interpretation of the gene's phenotype. For example, at least the *2, *3, and *17 should be included when genotyping for CYP2C19, while other additional variants are optional. This classification was based on the prevalence of these variants in different ethnic groups and their functional effect on enzyme activity and drug response (Pratt et al., 2018). Of note that one has to constantly review the literature for emergent variants or haplotypes such as the common CYP2C:TG haplotype, defined by rs2860840T and rs11188059G co-occurrence, that is associated with CYP2C19 increased enzyme activity, hence affecting metabolism of drugs such as sertraline and escitalopram (Braten et al., 2021; Braten et al., 2022).

3.3 Specifying genotyping strategies and methods

Herein, two strategic decisions should be made by the P&T committee in coordination with the molecular laboratory. The first decision is whether to genotype for the selected drug-gene pairs preemptively or reactively. The second is whether to test for one or only a few candidate gene variants or perform more extensive panel genotyping. The points to be considered include, in addition to funds or reimbursement matters, availability of in-house or reference laboratories, IT expertise, EHR interfaces capable of holding and interpreting genetic test results, and well-trained HCPs able to deal with genetic data.

3.3.1 Laboratory considerations

The medical institution may either establish, already have a certified genotyping laboratory, or refer to an outside reference laboratory to perform the PGx test. Such decisions mainly depend on availability of expertise, resources and funds as well as the extent of demand for PGx testing. The Genetic Testing Registry website (National Center for Biotechnology Information NCBI, 2012; Rubinstein et al., 2013) presents information on already available genetic and PGx tests in the United States. Also, the National genomic test directory (National genomic test directory, 2022) provides genomic tests commissioned by the National Health Services in the United Kingdom. One can search for a specific gene of interest and get a list of tests with details on purpose and coverage, validity, genotyping methodology, associated evidence for effectiveness, and contact laboratories with credentials.

Regardless of whether the lab is in-house or contracted, four primary standards must be considered (Vo et al., 2017). First, and as noted above, the pharmacogene(s)' selection should be relevant to the tested population, and feasible with the available technology being a candidate or panel genotyping. Second, there must be documented evidence of good laboratory practices such as the College of American Pathologists accreditation and Clinical Laboratory Improvement Amendments certification for the USA, the European co-operation for Accreditation for Europe (European co-operation for Accreditation, 2022), and the International Laboratory Accreditation Cooperation for the international level (International Laboratory Accreditation Cooperation, 2022), to ensure accuracy, reproducibility, sensitivity, and specificity of the assay performed with reference and reportable ranges (Bristol, 2002; Endrullat et al., 2016). Third, the format of lab reports should be designed to include simple stand-alone gene results with or without interpretative comments. These are integrated into the EHR or an online portal, if available, or simple paper reports. Finally, it is important to negotiate with reference laboratories outside the institution on cost or reimbursement models with the possibility for financial assistance or partnerships as applicable (Tuteja et al., 2022). In the USA, genetic testing companies may facilitate the process through contracting payers to reimburse patients who are required to undergo the test, or making special discounts for out-of-pocket payers. In addition, the growing body of genetic testing companies increases the competition among them leading to better offers for the consumer (Wolff and Wolff, 2018). In Europe, pricing policies are developed to restrict manufacturers' power in controlling genetic testing prices to ensure availability and protect consumers from exaggerated charges (Vozikis et al., 2016).

3.3.2 Preemptive *versus* reactive testing

Genes are stable, and genetic data remain unchanged with time; hence it is a practical call to genotype high-risk gene variants and store them in EHRs before a PGx drug is needed. This preemptive genetic testing approach saves critical time and allows HCPs to prescribe PGx medications directly when required. This approach

TABLE 3 Proposed non-exhaustive list and description of genetic allele variants for the clinical implementation of pharmacogenomics (PGx).

Gene	Allele	rsID	Variation type	Phenotype	MAF from 1,000 genomes with few exceptions[a,b]		
					African	Asian	Europe
ABCG2	c.421	rs2231142	SNV	Decreased function	0.0129	0.2907	0.0944
CYP2B6	*9	rs3745274	SNV	Decreased function	0.3744	0.2153	0.2356
	*18	rs28399499	SNV	No function	0.0825	0.0000	0.0000
	*26	rs3826711	SNV	Decreased function	0.0000	0.0050	0.0000
CYP2C19	*2	rs12769205	SNV	No function	0.1967	0.3125	0.1451
		rs4244285	SNV		0.1702	0.3125	0.1451
		rs58973490	SNV		0.0008	0.0000	0.0040
	*3	rs4986893	SNV	No function	0.0023	0.0556	0.000
	*4	rs12248560	SNV	No function	0.2352	0.0149	0.2237
		rs28399504	SNV		0.0000	0.0010	0.0010
	*8	rs41291556	SNV	No function	0.0008	0.0000	0.0030
	*9	rs17884712	SNV	Decreased function	0.0098	0.0000	0.000
	*10	rs6413438	SNV	Decreased function	0.0015	0.0000	0.0000
	*17	rs12248560	SNV	Increased function	0.2352	0.0149	0.2237
CYP2C9	*2	rs1799853	SNV	Decreased function	0.0083	0.0010	0.1243
	*3	rs1057910	SNV	No function	0.0023	0.0337	0.0726
	*5	rs28371686	SNV	Decreased function	0.0166	0.0000	0.0000
	*6	rs9332131	Indel	No function	0.0083	0.0000	0.0000
	*8	rs7900194	SNV	Decreased function	0.0530	0.0000	0.0020
	*11	rs28371685	SNV	Decreased function	0.0242	0.0000	0.0020
	*13	rs72558187	SNV	No function	0.0000	0.0030	0.0000
	*14	rs72558189	SNV	Decreased function	0.0000	0.0010	0.0000
	*16	rs72558192	SNV	Decreased function	0.0000	0.0010	0.0000
	*29	rs182132442	SNV	Decreased function	0.0000	0.0030	0.0010
	*31	rs57505750	SNV	Decreased function	0.0015	0.0000	0.0000
	*33	rs200183364	SNV	No function	0.0000	0.0010	0.0000
	*45	rs199523631	SNV	No function	0.0000	0.0000	0.0010
CYP2D6	*3	rs35742686	Indel	No function	0.0040	0.0000	0.0189
	*4	rs3892097	SNV	No function	0.0605	0.0020	0.1859
		rs28371703	SNV		0.0204	0.0010	0.1730
		rs28371704	SNV		0.0204	0.0010	0.1730
		rs1058172	SNV		0.0000[a]	0.0211[a]	0.1125[a]
	*5	PV00430	Whole gene deletion	No function	-	-	-
	*6	rs5030655	Indel	No function	0.0008	0.0000	0.0199
	*9	rs5030656	Indel	Decreased function	0.0008	0.0000	0.0258
	*10	rs1065852	SNV	Decreased function	0.1127	0.5714	0.2018
		rs1058164	SNV		0.6344[a]	0.7230[a]	0.5678[a]

(Continued on following page)

TABLE 3 (*Continued*) Proposed non-exhaustive list and description of genetic allele variants for the clinical implementation of pharmacogenomics (PGx).

Gene	Allele	rsID	Variation type	Phenotype	MAF from 1,000 genomes with few exceptions[a,b]		
					African	Asian	Europe
		rs1135840	SNV		0.6205[a]	0.7180[a]	0.5673[a]
	*14	rs5030865	SNV	Decreased function	0.0000	0.0099	0.0000
	*17	rs28371706	SNV	Decreased function	0.2179	0.0000	0.0020
		rs1058164	SNV		0.6344[a]	0.7230[a]	0.5678[a]
		rs16947	SNV		0.3398[a]	0.0300[a]	0.3181[a]
		rs1135840	SNV		0.6205[a]	0.7180[a]	0.5673[a]
	*21	rs1058164	SNV	No function	0.6344[a]	0.7230[a]	0.5678[a]
		rs16947	SNV		0.3398[a]	0.0300[a]	0.3181[a]
		rs1135840	SNV		0.6205[a]	0.7180[a]	0.5673[a]
	*29	rs61736512	SNV	Decreased function	0.1097	0.0000	0.0000
		rs59421388	SNV		0.1074	0.0000	0.0000
	*36	rs1065852	SNV	No function	0.1127	0.5714	0.2018
		rs1135822	SNV		0.0003[a]	0.0180[a]	0.0002[a]
		rs1135823	SNV		0.0000	0.0000	0.0010
	*40	rs72549356	Indel	No function	0.0091	0.0000	0.0000
	*41	rs28371725	SNV	Decreased function	0.0182	0.0377	0.9066
	*xN	-	Copy number	Increased function	-	-	-
CYP3A5	*3	rs776746	SNV	No function	0.3035[a]	0.7130[a]	0.9299[a]
	*6	rs10264272	SNV	No function	0.1543	0.0000	0.0030
	*7	rs41303343	Indel	No function	0.1180	0.0000	0.0000
CYP4F2	*3	rs2108622	SNV	Decreased function	0.0825	0.2143	0.2903
DPYD	*2A	rs3918290	SNV	Decreased function	0.0008	0.0000	0.0050
	*13	rs55886062	SNV	Decreased function	0.0000	0.0000	0.0010
	c.2846	rs67376798	SNV	Decreased function	0.0008	0.0000	0.0070
	c.1129-5923	rs75017182	SNV	Decreased function	0.0008	0.0000	0.0239
HLA-A	HLA-A*31:01	-	-	High-risk allele	-	0.0556	0.0104
HLA-B	HLA-B*15:02	-	-	High-risk allele	-	0.0667	-
	HLA-B*57:01	-	-	High-risk allele	-	0.0111	0.0729
	HLA-B*58:01	-	-	High-risk allele	0.0611	0.0444	0.0104
MT-RNR1	c.1555	rs267606617	SNV	Conformational change	-	0.0015[b]	-
	c.1095	rs267606618	SNV	-	-	0.0019[b]	-
NUDT15	*2	rs746071566	Indel	No function	0.0015	0.0476	0.0030
	*3	rs116855232	SNV	No function	0.0008	0.0952	0.0020
SLCO1B1	*5	rs4149056	SNV	No function	0.0136	0.1230	0.1610
	*9	rs59502379	SNV	No function	0.0408	0.0000	0.0000
	*14	rs11045819	SNV	Increased function	0.0598	0.0030	0.1441
	*15	rs2306283	SNV	No function	0.8177	0.7619	0.4026

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TABLE 3 (*Continued*) Proposed non-exhaustive list and description of genetic allele variants for the clinical implementation of pharmacogenomics (PGx).

Gene	Allele	rsID	Variation type	Phenotype	MAF from 1,000 genomes with few exceptions[a,b]		
					African	Asian	Europe
TPMT	*2	rs1800462	SNV	No function	0.0008	0.0000	0.0060
	*3A	rs1800460	SNV	No function	0.0030	0.0000	0.0278
		rs1142345	SNV		0.0666	0.0218	0.0288
	*3B	rs1800460	SNV	No function	0.0030	0.0000	0.0278
	*3C	rs1142345	SNV	No function	0.0666	0.0218	0.0288
UGT1A1	*6	rs4148323	SNV	Decreased function	0.0008	0.1379	0.0070
	*28	rs3064744	Indel: TA (8)	Decreased function	0.4266	0.1290	0.2922
	*36	rs3064744	Indel: TA (6)	Increased function	0.4266	0.1290	0.2922
	*37	rs3064744	Indel: TA (9)	Decreased function	0.4266	0.1290	0.2922
VKORC1	c. −1639	rs9923231	SNV	Decreased function	0.0545	0.8849	0.3877

MAF, minor allele frequency; **SNV**, single nucleotide variation; **Indel**, Insertion or Deletion.
[a]From Alfa Allele Frequency.
[b]From 14KJPN (Allele frequency panel of 14,129 Japanese individuals including the X chromosome).

protects the patient from trial and error with unwanted ADRs (Roden et al., 2018). Nevertheless, this strategy comes at a higher cost, necessitates complex technologies and EHR integration, and is typically not reimbursed as payers may not see the value of the test at the time of the request (Keeling et al., 2019; Haidar et al., 2022a). The other approach, the reactive point of care testing, is to order the genotyping test when a PGx drug is to be prescribed. Although less costly, this approach may not be timely since a drug prescription cannot be postponed in many cases, and the patient may suffer ADRs due to the wrong dosage or drug choice until the results are out (Nicholson et al., 2021; Haidar et al., 2022a). It is up to the institution to decide on which strategy is most suitable depending on funding and feasibility in the local context.

3.3.3 Candidate *versus* panel genotyping

As noted above, the medical institution may perform the planned PGx test in-house or refer to an outer reference laboratory. It should also strategize on choosing the candidate instead of panel genetic testing. Conversely, candidate genetic tests cover one or few specific gene variants related to a particular PK or PD pathway. The advantage of this type of test is that it is more accessible, less time-consuming, and less challenging to perform and interpret. It is mostly Polymerase Chain Reaction (PCR)-based or performed on small microarrays (Krebs and Milani, 2019; van der Lee et al., 2020; Verlouw et al., 2021). On the other hand, panel and genome-wide genotyping allow coverage of a more significant number of variants (Krebs and Milani, 2019; van der Lee et al., 2020; Verlouw et al., 2021). These tests may be ordered in patients taking multiple PGx actionable drugs or suspected to be prescribed various drugs based on age, comorbidities, or family history. This method is considered cost-effective since several genes can be genotyped together as one pool which decreases the cost per gene. For example, one may choose a panel of variants for drug transporters and CYP enzymes for a patient suffering from hypercholesterolemia to decide on the actionable rosuvastatin prescription. Since hypercholesterolemia is a risk factor for myocardial infarction, the panel can also cover variants for genes involved in the PK pathway of other cardiovascular drugs such as the antiplatelet clopidogrel. Panel tests are more challenging to deal with due to the large amounts of data they generate. In addition, this approach may not be practical in cases where the patient requires imminent treatment. Note that although panel testing includes many variants, one can decide to report only those associated with the prescribed drugs into the EHR. At the same time, the remaining results are stored in a separate database. This approach decreases the amount of data the HCP has to deal with, but at the same time, the data are readily available to be dispatched when a new PGx drug is added.

3.4 Reporting of results

Ideally, genotyping results should be reported in the patients' EHR. In case EHRs are available but are not compliant with genetic data, the IT team should upgrade the system to have the necessary features to receive such data. Yet, paper reports may also be considered (Cicali et al., 2022). Another important consideration is the language used to report genotyping data. For example, a report for CYP2C19 may state the result, such as that the genotype is CYP2C19*2, or may describe all evaluated genetic variants. The reporting discrepancies may lead to confusion in interpreting results from different laboratories. With the aim of standardising terms for allele functional status and inferred phenotype for the CPIC guidelines, Caudle et al. (2017) surveyed experts with diverse involvement in at least one area of PGx, and agreed on the following consensus terms: increased, normal, decreased, no, unknown, and uncertain

TABLE 4 List and description of few pharmacogenomic (PGx) clinical implementation programs and initiatives.

Description	Choice of drug-gene pairs	Genotyping strategies and methods	Results, EHR integration and CDS	Education
PMP: PERSONALIZED MEDICINE PROGRAM at the University of Florida Health since 2012 [1-3] https://precisionmedicine.ufhealth.org/about-us/				
➢ It builds and evaluates PGx information for clinical implementation	➢ Based on CPIC guidelines, genotyping of CYP2C19 for clopidogrel was initially launched, followed by TPMT for thiopurines IFNL3 for PEG-IFNα, CYP2D6 for opioids, CYP2D6 and CYP2C19 for SSRIs, and CYP2C19 for PPIs	➢ Involvement of the Pharmacy and Therapeutics Committee	➢ Hospital regulatory body leads the integration of relevant PGx results into EHR and CDS system	➢ Interactive learning opportunities focusing on review of evidence and development of clinical recommendations
➢ It also identifies and addresses common challenges		➢ Preemptive genotyping	➢ Rapid reporting of results into the EHR (Epic) after 2–3 days	➢ Education of target audience by provider group. Provision of material (printed and online) for clinicians and patients
	➢ Choice was also based on FDA product label, presence of no function genetic variants or common allele frequency, potential to prevent adverse drug events, available evidence supporting genotype-guided dosing recommendations, and physician request	➢ Life technologies Quant Studio Open Array technology. Chip-based genotyping	➢ Use of Best Practice Advisories (BPA) CDS system that provides interpretation and clinical recommendations based on patient's genetic results	➢ Development of a novel elective course for pharmacy students
				➢ Development of accredited post-graduate training programs in PGx
				➢ Publication of a newsletter titled "SNP.its"
PG4KDS: PHARMACOGENETICS FOR KIDS at the St. Jude Children's Research Hospital since 2011 [3-5] https://www.stjude.org/treatment/clinical-trials/pg4kds-pharmaceutical-science.html				
➢ It targets children with cancer	➢ Based on CPIC guidelines, genotyping for CYP2C19, CYP2D6, TPMT, and SLCO1B1 were initially chosen coupled with 12 high-risk drugs. After that, DPYD, UGT1A1, CYP3A5, CYP2C9, NUD15, RYR1, mt-RNR1, CACNA1S, G6PD, and CYP2B6 were genotyped, which resulted in therapeutic guidance for 66 drugs	➢ Creation of a subcommittee of the hospital Pharmacy and Therapeutics Committee for PGx oversight	➢ Test results are first displayed in a specialty flow sheet tab. Some are then moved to the EHR with phenotype description, interpretation, and implication	➢ Development of accredited post-graduate programs in clinical PGx
➢ It preemptively analyzes patients' DNA for a large number of gene variants, generates reports, and incorporates relevant PGx data in EHR coupled with CDS.	➢ Focus on drugs for children with cancer	➢ Launching of a research protocol with informed consent to implement preemptive genotyping strategy with integration into the EHR.	➢ Consultation notes are available for clinicians with basic PGx knowledge as a passive decision support tool	➢ Website includes presentations and publications on the implemented drug-gene pairs
		➢ Initially started with the Affymetrix DMET Plus assay, later moved to the right patient right drug (RPRD) diagnostic with the PharmacoScan array	➢ Results and consultations are available in the patient's online portal	
			➢ Active CDS alerts with relevant drug prescriptions	

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TABLE 4 (*Continued*) List and description of few pharmacogenomic (PGx) clinical implementation programs and initiatives.

Description	Choice of drug-gene pairs	Genotyping strategies and methods	Results, EHR integration and CDS	Education
PREDICT: PHARMACOGENOMICS RESOURCE FOR ENHANCED DECISIONS IN CARE AND TREATMENT at Vanderbilt University Medical Center since 2010 [3, 5, 6] https://www.vumc.org/predict-pdx/				
➢ It chooses drug-gene pairs, genotypes, filters, interprets, and incorporates PGx data and CDS in EHRs to be accessible for healthcare providers in routine care	➢ Based on CPIC guidelines, CYP2C19 was initially genotyped for clopidogrel followed by CYP2C9 and VKORC1 for warfarin therapy	➢ Involvement of the Pharmacy and Therapeutics Committee	➢ Results are entered by laboratory staff to the laboratory information system (Cerner Millennium Helix® module)	➢ Development of "My Drug Genome" website
	➢ Focus on drugs for cardiovascular diseases	➢ Preemptive genotyping	➢ Results of discrete variants are found on the EHR (Epic) as patient friendly version through My Health At Vanderbilt (MHAV)	➢ Support for the development of a Massive Open Online Course (MOOC) on PGx
		➢ TaqMan® chemistry-based platforms such as Oper QuantStudio™ 12K Flex Real-Time OpenArray Polymerase Chain Reaction (PCR) platform for more than 50 samples/day	➢ Application of end-to-end CDS system to help in interpretation of results and guidance in medication/dose selection	➢ Offering of a post-doctoral fellowship program and training in pharmacogenomics
			➢ Use of drug-gene interaction knowledge to interpret genotype-phenotype relation and linking of a specific CDS to a specific genetic result	
RIGHT: RIGHT DRUG, RIGHT DOSE, RIGHT TIME at the Mayo Clinic since 2013 [3, 5, 7] https://www.mayo.edu/research/centers-programs/center-individualized-medicine/research/clinical-studies/right-10k				
➢ It evaluates available PGx studies and guidelines	➢ Based on CPIC guidelines, genotyping of SLCO1B1 for simvastatin was initially done followed by CYP2C19 for clopidogrel, IFNL2 for interferon, CYP2D6 for tramadol, tamoxifen and codeine, HLA-B*1,502 for carbamazepine and abacavir, and TPMT for thiopurines	➢ Involvement of the pharmaceutical formulary committee in approving drug-gene pairs and incorporation of results with CDS.	➢ Storage of molecular diagnostic laboratory results in EHR.	➢ The CDS rules provide information on drug-gene pair at point of care as a "Just in Time" support system
➢ It genotypes and incorporates PGx data and CDS into EHR to be accessible for healthcare providers	➢ Choice was also based on commonly prescribed drugs containing actionable PGx variants, FDA list of PGx biomarkers, PharmGKB list of genes and drugs, Indiana University Drug Interactions website, articles published on the subject of PGx, and current PGx tests offered by the Mayo Clinic's Department of Laboratory Medicine and Pathology	➢ PGx implementation model following this sequence: Institutional leadership support, Pharmacogenomics governance, Clinical approval, Laboratory results, Pharmacogenomics education, Pharmacogenomics knowledge, CDS-EHR implementation, and long-term maintenance	➢ Development and maintenance of CDS rules that involve conversion of variants to standard notation and interpretation, workflow analysis, and data mapping	➢ Information and interpretation of PGx testing is available for patients through "Online Patient Services account"
		➢ Development of Mayo Clinic Biobank Community Advisory Board (CAB) for recruitment and consenting patients	➢ CDS rules are implemented for interpreting results, prescribing decisions, and providing actionable alert messages	➢ Development of "Ask Mayo Expert" for patient education

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TABLE 4 (*Continued*) List and description of few pharmacogenomic (PGx) clinical implementation programs and initiatives.

Description	Choice of drug-gene pairs	Genotyping strategies and methods	Results, EHR integration and CDS	Education
		➢ Preemptive research genotyping through the RIGHT protocol	➢ Active CDS alerts are developed in the computerized physician order entry (CPOE) applications	➢ Establishment of grand rounds, presentations, online modules, videos, brochures, and links to results through the patient's portal
		➢ Use of PGRN-Seq technique		➢ Offering of a post-doctoral fellowship program and training in PGx
The 1200 PATIENT PROJECT at the University of Chicago Center for Personalized Therapeutics since 2011 [3, 5, 8] https://cpt.uchicago.edu/1200-patients-project/				
➢ It assesses the effectiveness and feasibility of applying preemptive PGx testing in clinical settings	➢ Large number of germline polymorphisms for outpatient medical care	➢ Research study targeting 1,200 patients for the implementation of preemptive genotyping with informed consent	➢ Results, interpretation and education are available through research web-portal or genomic prescribing system (GPS)	➢ Development of "YourPGx Portal"
➢ It also evaluates the impact of using PGx results on prescription decisions and patients' outcome	➢ Participants should be taking 1 to 6 prescription medications of interest	➢ Use of 'ADME pharmacogenomics panel' and custom Sequenom panel		➢ Offering of a post-doctoral fellowship program and training in PGx
	➢ Choice of genes based on published clinical evidence for their PGx role			
U-PGx: UBIQUITOUS PHARMACOGENOMICS Consortium in Europe since 2016 (The Netherland, United Kingdom, Germany, Sweden, Austria, France, Italy, Spain, Greece, Slovenia) [3] https://uPGx.eu/				
➢ It evaluates the cost-effectiveness and impact of preemptive PGx implementation in Europe on patient outcome by conducting 'The Preemptive Pharmacogenomic testing for Prevention of adverse drug reactions (PREPARE)' study	➢ Panel of 50 variants in 13 pharmacogenes that have actionable drug-gene interaction based on the Dutch Pharmacogenetics Working Group (DPWG) guidelines	➢ Research study (PREPARE) on the preemptive implementation of a panel of pharmacogenes covering several therapeutic areas	➢ Medication Safety Code system: "Safety-Code card" and Genetic Information Management Suite for physicians	➢ Established E-learning PGx programs for healthcare providers
	➢ The 13 genes evaluated are: CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A5, DPYD, F5, HLA- B*5701, SLCO1B1, TPMT, UGT1A1, VKORC1	➢ Use of SNPline platform		
	➢ For patients being started on a drug of interest that has clinically relevant genetic interaction with the genes mentioned. The chosen drugs are very similar to the ones listed in Table 2			

EHR, electronic health records; CDS, clinical decision support.

1. Johnson, J.A., et al., Institutional profile: University of Florida and Shands Hospital Personalized Medicine Program: clinical implementation of pharmacogenetics. Pharmacogenomics, 2013. **14** (7): p. 723-6.

2. Cavallari, L.H., et al., Institutional profile: University of Florida Health Personalized Medicine Program. pharmacogenomics, 2017. **18** (5): p. 421-426.

3. Van der Wouden, C.H., et al., Implementing Pharmacogenomics in Europe: Design and Implementation Strategy of the Ubiquitous Pharmacogenomics Consortium. clin pharmacol ther, 2017. **101** (3): p. 341-358.

4. Haidar, C.E., et al., Advancing Pharmacogenomics from Single-Gene to Preemptive Testing. annu rev genomics hum genet, 2022. **23**: p. 449-473.

5. Luzum, J.A., et al., The Pharmacogenomics Research Network Translational Pharmacogenetics Program: Outcomes and Metrics of Pharmacogenetic Implementations Across Diverse Healthcare Systems. clin pharmacol ther, 2017. 102 (3): p. 502-510.

6. Liu, M., et al., A Tutorial for Pharmacogenomics Implementation Through End-to-End Clinical Decision Support Based on Ten Years of Experience from PREDICT., clin pharmacol ther, 2021. 109 (1): p. 101-115.

7. Bielinski, S.J., et al., Preemptive genotyping for personalized medicine: design of the right drug, right dose, right time-using genomic data to individualize treatment protocol. Mayo Clin Proc, 2014. 89 (1): p. 25-33.

8. O'Donnell, P.H., et al., The 1,200 patients project: creating a new medical model system for clinical implementation of pharmacogenomics. Clin Pharmacol Ther, 2012. 92 (4): p. 446-9.

function for allele functional status of all genes; ultrarapid, rapid, normal, intermediate, and poor metabolizer for drug-metabolizing enzymes; increased, normal, decreased, and poor function for the phenotype of transporters; and positive or negative for high-risk genotype status such as for HLA-B. It is hence advisable to include an interpretation of the phenotype associated with the resultant genotype, coupled with a recommendation on what to do next with the drug to be prescribed using language from CPIC or other guidelines. In addition, and if possible, CDS systems should be incorporated to guide the HCP into an informed decision based on genetic data (Caraballo et al., 2017; O'Donnell et al., 2014).

CDS systems can be passive or active. The first generates alerts that are stored in the patient's EHR for use when needed, while the latter spontaneously delivers alerts pre- and/or post-PGx testing (Hicks et al., 2016; Haidar et al., 2022a). Passive CDS systems require the HCP to remember at the point of care to look for the potential drug-gene interaction, and find the PGx test results in the EHR if available (Haidar et al., 2022a). For active CDS systems, the pre- PGx test alert is triggered when an HCP prescribes an actionable drug at the point of care, and the patient lacks previous genetic test results (Bell et al., 2014). The alert is either displayed directly on the computer screen or by email to direct the HCP to order a genotyping test (Bell et al., 2014). The post- PGx test alert is triggered when a HCP prescribes an actionable drug that coincides with already available high-risk gene data (Bell et al., 2014; Haidar et al., 2022a). The alert provides phenotype interpretation, possible drug-gene interaction, if any, and recommended actions, such as changing the drug or dose, or monitoring (Bell et al., 2014; Haidar et al., 2022a).

4 Recommendations for the further enhancement of pgx implementation in clinical practice

Some strategies should be put in place to frequently evaluate emerging evidence, continuously audit and evaluate the progress and performance of the program, and integrate research for ethical, legal and social issues (Pirmohamed, 2023). In addition, there is a need to educate HCPs and patients, and to push for more tests' reimbursement as detailed below.

4.1 Education

HCPs and patients are on the receiving end of the clinical PGx implementation process. HCPs, be they physicians or pharmacists, are responsible for the return of results to patients. HCPs and patients are both accountable for reporting back on the outcome of the PGx-guided prescription. They need education to enhance their use and understanding of the whole practice.

4.1.1 Healthcare providers

Survey data have consistently shown that HCPs, despite being generally aware of the importance of PGx and having a positive attitude towards PGx's ability to improve drug therapy and reduce side effects, few have ordered or recommended PGx testing (Stanek et al., 2012; Abou Diwan et al., 2019; Algahtani, 2020). This lag in PGx clinical implementation is primarily related to the lack of formal education about PGx testing in medical school and postgraduate studies (Stanek et al., 2012; Algahtani, 2020). For instance, a survey among HCPs on PGx education in Europe showed that 83.3% of the participants still lack PGx expertise (Just et al., 2017). Another global survey study was recently done to evaluate the current status of PGx education in medical and pharmacy study programs (Karas Kuzelicki et al., 2019). Results showed that 13.4% had no PGx education among the recruited participants, 19.6% took PGx as an independent elective, and only 10.3% had PGx as a mandatory subject. These results were congruent with survey data collected over a decade ago (2005) and led to the recommendation that PGx should be taught as an integral part of pharmacology curricula (Gurwitz et al., 2005; Karas Kuzelicki et al., 2019). These recommendations are crucial, knowing that education and training increase physicians' confidence to request PGx tests or use such test results if already available before prescribing drugs (Luzum and Luzum, 2016).

Undergraduate education that considers PGx as foundational content can hence contribute to the education of HCP graduates for the integration of PGx into clinical care. Also, training during fellowships or residencies, graduate and postgraduate programs, or certificates can address the PGx knowledge gap. As such, accreditation standards have been developed by the American Society of Health-System Pharmacists for postgraduate year two residence pharmacists to include clinical PGx training (Haidar et al., 2022b).

Besides, continuous education activities are essential to staying up to date. These include just in-time education such as active CDS systems, and PGx programs that provide on-site services, dedicated webpages, and messages to the clinician's inbox (Freimuth et al., 2017; Williams, 2019). Also, one can build on the already available educational resources, such as those by CPIC. In addition, the National Human Genome Research Institute has supported an Inter-Society Coordinating Committee to develop educational resources aiming to improve the PGx education of HCPs (National Human Genome Research Institute, 2021; Haidar et al., 2022a).

4.1.2 Patients

Patients' awareness and education are essential drivers for the success of PGx implementation. Available studies suggest that patients have a positive attitude toward PGx implementation and believe in its ability to predict the correct dose, medication efficacy, mild or serious side effects, and explain the family history of medication toxicity (Nielsen and Moldrup, 2007). The general public can also understand specific genetic terminology, yet people cannot comprehend underlying concepts and how this may affect their health (Lea et al., 2011; Haga et al., 2014).

To address this gap, PGx education may be provided through technological tools such as interactive webpages, educational videos, and telehealth sessions (Hoffman et al., 2014; Dunnenberger et al., 2015). Moreover, written reports should be user-friendly. They can include summaries of genetic results in a tabular or graphical format

such as infographics (e.g., human icon drawn as running motion representing rapid metabolizer) and icon arrays (e.g., shaded figures reflecting the proportion of affected out of total) (Galesic et al., 2009; Sinayev et al., 2015). Also, simple drawings of pie charts, risk labels, and tri-color-coding systems for risk assessment (red, yellow/orange, and green for high, moderate, and average risk, respectively) (Haga, 2017) are examples of patient-friendly formats. These methods can generate more confidence in patients toward PGx implementation. Finally, adequate counseling should be provided through written reports or in-person contact by well-trained HCPs or genetic counselors if available (Haga, 2017; Haidar et al., 2022a).

4.2 Reimbursement considerations

The cost of PGx testing varies between companies and platforms, with the cost of single-gene tests ranging from 100$ to 500$, while the price of a multigene panel test may reach double that of the single-gene test (Anderson et al., 2020; Haidar et al., 2022a). Although single-gene test costs less, patients may require the prescription of several actionable drugs, whereby in this case, multigene panel testing becomes more cost-effective. Nevertheless, most payers are still reluctant to reimburse multigene panel tests due to the lack of evidence of clinical utility for preemptive panel testing (Keeling et al., 2019). Even though the clinical utility of reactive testing using single-gene tests is easier to obtain compared to preemptive multigene panel testing, its reimbursement is still a barrier (Haidar et al., 2022a).

However, reimbursement of PGx in the US may be forthcoming (Empey et al., 2021). Lately, local coverage determinations for the Molecular Diagnostic Services program were established based on earlier decisions made by payers (public and private). They expanded the coverage for some Medicare Administrative Contractors that cover molecular diagnostic tests (US Centers for Medicare and Medicaid Services, 2020). Accordingly, for Medicare patients, local coverage determinations are indicated for PGx tests related to medications that are medically necessary, appropriate, and approved for the patient's condition and have clinically actionable drug-gene interaction defined by the FDA and CPIC (US Centers for Medicare and Medicaid Services, 2020). Moreover, payer reimbursement policies are evolving, and the availability of specific criteria for PGx testing may increase the probability of coverage (Keeling et al., 2019). For example, the American Medical Association has created several Current Procedure Terminology codes for single-gene PGx tests to detect specific gene variants that impact drug therapy. These codes result in more specific documentation that may increase the chance of PGx test coverage (Hefti and Blanco, 2016). Also, the establishment and demonstration of evidence that PGx improves clinical outcomes, and finding value for PGx testing, as reflected by helping HCPs to decide on therapy for a specific population, may increase the possibility of PGx test reimbursement (Weitzel et al., 2019).

In Europe, the reimbursement systems differ among individual countries (Payne and Annemans, 2013). For the sake of illustration in the Netherlands, all citizens should have a basic healthcare insurance that includes coverage for PGx tests that are ordered to explore causes of ADRs. Moreover, optional reimbursement packages for PGx screening are provided by some healthcare insurers (van der Wouden et al., 2020). In addition, The "G-standaard" Dutch drug database offers information, guidance and standards that are used by different parties in healthcare including health insurers to enhance the infrastructure for national testing programs (Thornley et al., 2021). Finally in England, the National Health Services Genomics Medicine Service that aims to provide genetic services equally among patients is leading innovative projects to allow integration of genetic testing into routine healthcare through supporting research, adequate planning, and reimbursement (Robinson, 2022). Interestingly, a system for reimbursement of PGx testing was suggested whereby it is proposed for medical authorities to develop a "positive medical device" list to control the cost in the market, and impose reimbursement by insurance companies (Vozikis et al., 2016).

Besides coverage by payers, some commercial laboratories provide reimbursement on their panel-based testing by income-based sliding scale payment method, patient assistance programs, or help the patient navigate the reimbursement process. Also, the PGx test can be initially covered or supplemented by institutional support or research funding (Luzum et al., 2017; Cicali et al., 2022).

5 Conclusion

This article proposed an approach to designing and implementing clinical PGx in the hospital setting. After test authorization or requirements for testing by the government or drug regulators, putting the plan into action involves several stakeholders, with the hospital leadership supporting the process and communicating with payers, the P&T committee leading the process in collaboration with the hospital laboratory and IT department, and HCPs ordering the test, understanding the results, making the appropriate therapeutic decisions, and explaining them to the patient. We concluded by recommending strategies to further advance the implementation of PGx in practice, such as the need to educate HCPs and patients and to push for more tests' reimbursement. The reader can refer to Table 4 and learn from the experience of other institutions that have been implementing PGx for years for clinical and or research purposes and adapt some of their approaches concerning the choice of drug-gene pairs, genotyping strategies and methods, integration of results and reports, and educational practices. Several barriers and schemes should be considered before implementing clinical PGx on a big scale (Swift, 2022; Tuteja et al., 2022; Pirmohamed, 2023).

Author contributions

DK, RA and NKZ conceptualized and wrote the first draft of the manuscript. DK gathered and tabulated data from the guidelines and drug labels. AW, AD and IC commented on the concepts and content, and contributed to the writing. All authors approved the final version of the submitted work. All authors read and approved the final manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Supplementary material

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Impact of CYP2D6 genotype on opioid use disorder deprescription: an observational prospective study in chronic pain with sex-differences

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Introduction: Opioid deprescription is the process of supervised tapering and safe withdrawal when a potentially inappropriate use is detected. This represents a challenge in chronic non-cancer pain (CNCP) patients who may respond differently to the procedure. Our aim was to analyze the potential impact of CYP2D6 phenotypes and sex on the clinical and safety outcomes during an opioid use disorder (OUD) tapering process.

Methods: A prospective observational study was conducted on CNCP ambulatory OUD patients (cases, n = 138) who underwent a 6-month opioid dose reduction and discontinuation. Pain intensity, relief and quality of life (Visual analogue scale, VAS 0–100 mm), global activity (GAF, 0–100 scores), morphine equivalent daily dose (MEDD), analgesic drugs adverse events (AEs) and opioid withdrawal syndrome (OWS, 0–96 scores) were recorded at basal and final visits. Sex differences and CYP2D6 phenotypes (poor (PM), extensive (EM) and ultrarapid (UM) metabolizers based on CYP2D6*1, *2, *3, *4, *5, *6, *10, *17, *41, 2D6*5, 2D6 × N, 2D6*4 × 2 gene variants) were analyzed.

Results: Although CYP2D6-UM consumed three-times less basal MEDD [40 (20–123) mg/day, p = 0.04], they showed the highest number of AEs [7 (6–11), p = 0.02] and opioid withdrawal symptoms (46 ± 10 scores, p = 0.01) after deprescription. This was inversely correlated with their quality of life (r = –0.604, p < 0.001). Sex-differences were evidenced with a tendency to a lower analgesic tolerability in females and lower quality of life in men.

Discussion: These data support the potential benefits of CYP2D6-guided opioid deprescription, in patients with CNCP when OUD is detected. Further studies are required to understand a sex/gender interaction.

KEYWORDS

CYP2D6, sex-differences, opioid use disorder, deprescription, chronic pain, pharmacogenetics

1 Introduction

The current international analgesic landscape is characterized by a significant global increase in the use of prescription opioid (Upp and Waljee, 2020; Di Gaudio et al., 2021). In fact, 15.2% of the adult Spanish population admits having used opioid analgesics, at some point in their lives (Spanish Observatory on Drugs and Addictions OEDA, 2021), with observed differences in the use and the presence of any opioid use disorder (OUD) between sexes (McHugh et al., 2018). This problematic opioid use has resulted in formulation of practice-specific guidelines as a mechanism to curb current trend (National Academies of Sciences and and Medicine, 2017). In this context, research shows that patients in severe pain despite use of high-dose opioids may experience significant improvement in pain relief and functioning, when their opioid is tapered to a lower, safer dose (Kahan et al., 2011), improving adherence and reducing drug-seeking behaviors (Becker et al., 2018).

Current evidence suggests potential genetic factors that could be used to predict one's risk of opioid misuse or a problematic use (Singh et al., 2021), harmful (Muriel et al., 2019) or addictive potential (Linares et al., 2014). There is some evidence suggesting CYP2D6 enzyme, responsible for the metabolism of tramadol, codeine and oxycodone, may be more efficient at ultra-rapid metabolizer (UM) synthesizing endogenous opioids (Zahari and Ismail, 2014), experience quicker and higher systemic levels of the active metabolites and therefore, to require lower analgesic doses (Candiotti et al., 2009). However, UM subjects will be prone to higher mu-opioid-related toxicity and a higher risk of adverse events (AEs) (Lopes et al., 2020). In contrast, CYP2D6 poor metabolizers (PMs) would tend to have lower levels of the active metabolites (Haufroid and Hantson, 2015), which may result in reduced analgesic efficacy (Lötsch et al., 2004; Zahari and Ismail, 2014). This could have special impact for females who generally exhibit a lower opioid tolerability in comparison to males (Planelles et al., 2020), which can be turned into differences in opioid´s clearance (Anderson, 2008). Here, scarce data on the effect of sex on the CYP2D6 activity exist, and except for some data related to menstrual cycle influence (Tamminga et al., 1999), explicit recommendations derived through a validated process have not yet been formulated (He et al., 2015).

In this sense, there is increasing evidence in humans and laboratory animals for sex differences in processes of reward and addictive behavior, withdrawal, craving, and relapse due to psychostimulants and opioids (Becker and Chartoff, 2018). In fact, women are more likely to refer and be diagnosed with acute and chronic pain and to be prescribed these drugs in significantly greater numbers than men (Goetz et al., 2021). Although several reports have documented risk factors for opioid use following treatment discharge, yet few have assessed sex differences in long-term opioid use in chronic non-cancer pain (CNCP) management (Cragg et al., 2017; National Academies of Sciences and and Medicine, 2017; Davis et al., 2021).

The primary goal of the present study was to evaluate the impact of CYP2D6 phenotypes and sex influence on OUD deprescription ambulatory CNCP patients. As a primary hypothesis, it was considered that CYP2D6-UM metabolizers would show a different clinical outcome pattern when compared to the other groups, as would be also observed between sexes.

2 Materials and methods

2.1 Study design and selection of participants

This manuscript adheres to the applicable STROBE guidelines. This prospective observational pharmacogenetic study followed the current Declaration of Helsinki and European Medicines Agency Guidelines for Good Clinical Practice and was approved by the Ethics Committee of The General University Hospital of Alicante. Written informed consent was obtained from all participants prior to their inclusion in the study.

All the CNCP consecutive patients with confirmed OUD who underwent a 6-month opioid deprescription (cases, n = 138) by clinical practice at the Pain Unit (PU, General University Hospital of Alicante, Alicante, Spain) from May 2013 to May 2019 were included under the inclusion criteria prior to deprescription: 1) patients aged 18 years or older; 2) with CNCP and long-term opioid use (>6 months); 3) OUD diagnosis according to diagnostic DSM-5 criteria (American Psychiatric Association, 2013) as confirmed by a psychiatrist; and 4) informed consent granted. All the cases were followed-up prospectively for opioid dose reduction and discontinuation. A control group of 231 participants who had previously participated in observational studies from the same setting which were under opioids for chronic pain and no OUD suspicion (Margarit et al., 2019) was included to explore potential differences in terms of sociodemographic, clinical, pharmacological and CYP2D6 phenotypes in comparison to the cases.

2.2 Description procedure

The deprescription program was designed, established and executed according to national and international guidelines (Fernández-Miranda, 2007). OUD was defined as a problematic pattern of opioid use that causes significant impairment or distress according to the criteria in the DSM-5 (American Psychiatric Association, 2013). Here, a monitored opioid rotation to tramadol/buprenorphine together with the tapering process (progressive opioid withdrawal through a rotation with dose-reduction and control of any withdrawal symptoms) was conducted through consecutive clinical visits along 6 months (Muriel et al., 2019; Muriel et al., 2018). Depending on the patients' clinical status they were fully rotated to buprenorphine/tramadol from their basal prescriptions or stayed on their basal prescriptions but lower doses with tramadol as rescue medication. Basal MEDD was ideally 20%–30% reduced at each clinical visit (follow-up visits (1, 2 weeks, 1 and 3 months) and a final visit at 6 months) starting with the total withdrawal of quick-release opioids. Any precipitated opioid withdrawal symptom was carefully monitoring at each clinical visit. Effectiveness, as primary outcome, was considered when neither OUD nor any aberrant opioid use behavior was observed together with a morphine equivalent daily doses (MEDD) reduction minimum of 30% from basal levels - as a clinically meaningful reduction in dose (Perez et al., 2020) - or opioid discontinuation.

2.3 Clinical data collection

Demographic characteristics (age, sex) and clinical variables were collected using validated questionnaires and scales completed at each of the patients' visit. Pain intensity and relief were measured using the Visual Analogue Scale (VAS) (McCormack et al., 1988). Both VAS scales consist of a 100 mm horizontal line ranging from 0 (lowest) to 100 mm (highest). Similarly, VAS-EuroQol Scale (EQ) was used for quality of life assessment (EuroQol, 1990). Opiate Withdrawal Scale (OWS, 0–96 scores) is a questionnaire composed of 32 common symptoms in opioid withdrawal patients (Bradley et al., 1987) rated using scores of 0 (absent) to 3 (severe). The Global Assessment of Functioning (GAF, 0–100 scores) scale was used to assess patient's psychological, social, and work activity independently from the activity alterations caused by physical limitations. Higher score meaning a better level of activity and life (Jones et al., 1995).

2.4 Drug use and adverse events

Opioid and co-adjuvant medications were strictly prescribed by clinical judgement by the physician without any experimental decision. Use of opioid and non-opioid analgesics, NSAIDs, antidepressants (duloxetine), anxiolytics (benzodiazepines) and neuromodulators (pregabalin and gabapentin) was obtained from EHRs. MEDD were estimated using the available opioids equivalent doses (Pergolizzi et al., 2008) and classified as being low (MEDD<100 mg/day) or high (MEDD≥100 mg/day), given the potential increased dose-dependent side-effects (Chapman et al., 2010; Dunn et al., 2010). In addition, MEDD was calculated and analyzed separately in those patients with use of CYP2D6-mediated opioids (oxycodone, hydrocodone, tapentadol, codeine and tramadol).

To assess the tolerability, a questionnaire with the list of the most frequently occurring AEs (according to the opioids' summary of product characteristics, including "very common" and "common" listings) (Boiarkina and Potapov, 2014) and a blank field to add any other, was used to record patients' occurrence of AEs (Barrachina et al., 2021). In addition, all ADRs (Wisher, 2012) were collected and classified using the Medical Dictionary for Regulatory Activities (MedDRA, version 20.0) and the Preferred Terms.

2.5 *CYP2D6* genotyping

Approximately 2 mL of saliva was collected in PBS containing tubes. Genomic DNA was extracted using an E.N.Z.A. Forensic DNA Kit (Omega bio-tek), according to the manufacturer's instructions. Genetic analysis was based in usual PCR-methods following the instructions of the Consortium of Pharmacogenetics (CEIBA) and the pharmacogenomics iberoamerican network (RIBEF) for the analysis of samples. XL-polymerase chain (XL-PCR) analysis was used for identification of duplications and deletions (Dorado et al., 2005). These XL-PCR amplifications were carried out in a Mastercycler 384 (Eppendorf, AG, Hamburg, Germany). After the genotype was stablished, the different variants were converted to an Activity Score (AS), which indicated the enzyme's activity level (null, reduced, normal, increased) (Gaedigk et al., 2008). Presence of SNP *3, *4, *5 or *6 represents an AS of 0, which means a null enzyme activity. Variants *10, *17 and *41 are associated with an AS of 0.5 and *1, *2 and *35 with an AS of 1, representing reduced and normal enzyme activity levels, respectively. Presence of duplications *1xN, *2xN or *35xN suppose an increased enzyme activity level (AS = 2). According to previous classifications, if the AS resulting from the combination of both alleles was zero, the subject was considered as PM; if ranges from 0.5 to 2 as EM; and above 2 as UM (Naranjo et al., 2016).

2.6 Statistical analyses

Based on the observational prospective nature of the study and to the inclusion limited by the low frequency of patients with an OUD, a convenience sample was proposed. As an estimated prevalence of 3.2% of OUD was detected in our setting (Muriel et al., 2018). Out of an average of 915 patients/year who visit our PU, 30 potentially eligible subjects per year were expected. Due to the missing or refusing to participate (almost 20%), approximately 24 patients were expected annually. To complement the analysis, a control group from our previous study was proposed. As the condition/event (OUD) is infrequent (<10% prevalence), a complete series of controls was included to achieve a superior number of controls (ratio 2:1).

Data distribution was analyzed using Kolmogorov-Smirnov normality test. Quantitative parametric data are presented as mean (SD) while median (IQR) was used for non-parametric data and discrete variables. Categorical data are expressed by percentages. Comparisons of continuous data between two groups were conducted using a *t*-test for parametric data, meanwhile for non-parametric, U Mann-Whitney test was used. When analyzing categorical data between two groups, Fischer's exact test was performed. For the analyses of the three metabolic phenotypes, ANOVA test was performed for parametric continuous data and Kruskal-Wallis for non-parametric. In this case, Chi-square test was used for categorical analyses. *t*-test and/or U Mann-Whitney (for PM vs. EM/UM, EM vs. PM/UM and UM vs. PM/EM) were performed too. Gene by sex interaction was explored by invoking a regression model. All the obtained variables included a separate description and analysis by sex.

The Pearson correlation coefficient (r) and its 95% confidence intervals (CI) were calculated to analyze the correlation between opioid withdrawal and quality of life. Two groups (subjects included between 2013–2015 and 2016–2019) were compared to determine if deprescription outcomes changed over time. The MEDD difference between groups was expressed using the Hodges-Lehmann estimator shift with the 95% CI. In the assumption of missing completely at random, complete case (or available case) analysis was performed. A $p \leq 0.05$ was considered statistically significant. In all cases, multiple testing was adjusted using Bonferroni correction. All statistical analyses were carried out using R (3.2.0 version) software.

3 Results

A total of 138 patients (65% female) with an OUD were recruited and enrolled in the ambulatory opioid deprescription. Fifteen percent (n = 21) of the patients were lost to follow-up (n = 18 did not attend follow-up visits, n = 2 no biological

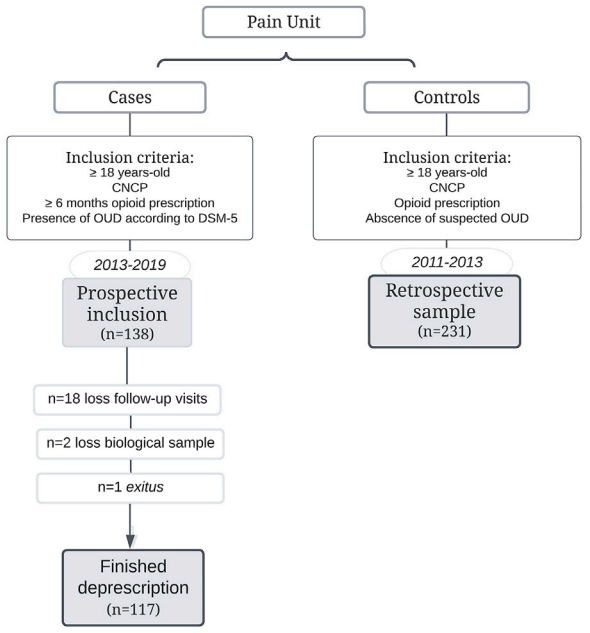

FIGURE 1
Flow Chart of patients with OUD (cases) along 6 years at Pain Unit and the control group included in the study.

samples, and $n = 1$ death due to intestinal pneumatosis) with 117 (66% female), of them completing the program. Data from a total of 231 subjects (64% female) were included as a control group (Figure 1).

At basal visit, cases showed a moderate basal chronic VAS pain intensity (60 (27) mm) and quality of life [45 (24) mm], with mild relief [37 (29) mm] and a mean of [32 (19) OWS scores]. No differences based on the inclusion period or between visits during deprescription were found in these outcomes. Here, patients evidenced "some mild symptoms or difficulties in social, interpersonal relationships or occupational functioning, but generally functioning pretty well" due GAF 71 (15) scores.

Cases were a mean of almost 10 years younger [54 (13) vs. 63 (14) years, $p < 0.001$], with a higher basal pain relief [37 (29) vs. 18 (13) mm, $p < 0.001$] probably due to a higher MEDD [120 (80–200) vs. 40 (0–82) mg/day, $p < 0.001$, difference Hodges-Lehmann: −80; 95% CI of the difference (−90 to −58)] at basal visit (Table 1).

3.1 Opioid deprescription

Clinical and pharmacological data of the total case population and classified by the CYP2D6 metabolic phenotypes is shown in Table 2.

Opioid deprescription was effective in 76% of the cases with a 42% of opioid discontinuation after tapering without differences due to sex. Total median MEDD was 67% significantly reduced with a final consumption of 40 (0–80) mg/day [$p < 0.001$, difference Hodges-Lehmann: −80 (−83 to −40)]. In consonance, the percentage of patients with a high MEDD level (>100 mg/day) decreased significantly from 55% to 27% ($p < 0.001$) without differences due to sex. Interestingly, cases included in later time period (2016–2019) showed a significant lower final MEDD [0 (0–80) mg/day] compared to those included in early time-period (2013–2015) [60 (0–160) mg/day, $p = 0.02$] (Supplementary Table S1).

3.2 *CYP2D6* phenotype

Metabolic CYP2D6 phenotypes were classified as 6% PM, 85% EM and 9% UM according to their genotype without differences in frequency between sexes (females 6% PM, 84% EM and 78% UM) or compared with the control group (5% PM, 89% EM and 6% UM). Allelic frequencies of *CYP2D6* variants can be seen in Supplementary Table S2.

Here, UM phenotypes showed a significantly lower three-times MEDD compared to PM-EMs [40 (20–123) vs. 123 (80–226), $p = 0.04$, difference Hodges-Lehmann: −63 (−140 to 0)]. However, when only CYP2D6 metabolism mediated opioids were selected,

TABLE 1 Sociodemographic, clinical, pharmacological and tolerability variables in cases (basal visit) and controls.

Basal visit	Cases ($n = 138$)	Controls ($n = 231$)	p-value[†]
Sex (female, %)	65	64	1.00
Age (years, mean (SD))	54 (13)	**63 (14)****	**<0.001**
Pain Intensity (VAS, 0–100 mm, mean (SD))	60 (27)	56 (31)	0.09
Pain relief (VAS, 0–100 mm, mean (SD))	**37 (29)****	18 (13)	**<0.001**
Quality of life (EQ, 0–100 mm, mean (SD))	45 (24)	45 (14)	1.00
Total MEDD (mg/day, median (IQR))	**120 (80–200)****	40 (0–82)	**<0.001**
AEs (median (IQR))	**5 (2–8)***	3 (1–6)	**0.03**
ADRs (%)	13	21	0.07

[†]Cases vs. controls comparisons using using *t*-test and U Mann-Whitney test for continuous parametric and non-parametric data, respectively, and Fisher's exact test for categorical data (significant $p < 0.05$ in bold).
*$p < 0.05$, **$p < 0.01$ (highest value in bold). VAS, visual analogue scale; EQ, VAS, EuroQol Scale (0–100 mm); AEs, adverse events; ADRs, adverse drug reactions; IQR, interquartile range, expressed in parenthesis as P25 and P75.

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TABLE 2 Demographic and pharmacological variables, in total population and classified by *CYP2D6* metabolic phenotype.

Variables		Cases (basal, *n* = 138; final, *n* = 117)	*CYP2D6* phenotype			*p*-value[†]
			PM (*n* = 7, 6%)	EM (*n* = 98, 85%)	UM (*n* = 10, 9%)	
Age [years, mean (SD)]		54 (13)	47 (12)	54 (13)	59 (14)	0.17
Sex (female, %)		65	71	65	80	0.61
Deprescription Responder (%)		76	80	76	89	0.66
Final opioid use (%)		58	80	55	56	0.55
Total MEDD [mg/day, median (IQR)]	*Basal*	120 (80–200)	120 (60–233)	123 (80–229)	**40 (20–123)**[+]	0.11
	Final	**40 (0–120)***	40 (7–65)	**40 (0–120)***	80 (0–150)	0.92
CYP2D6 opioid mediated MEDD [mg/day, median (IQR)]	*Basal*	40 (6–100)	40 (6–100)	40 (6–100)	40 (6–100)	0.81
	Final	**20 (0–43)****	20 (0–43)	**20 (0–43)****	20 (0–43)	0.64
High MEDD (>100 mg/day) (%)	*Basal*	55	60	59	22	0.10
	Final	**27** **	0	**30****	33	0.33
Pain Intensity [VAS, 0–100 mm, mean (SD)]	*Basal*	60 (27)	63 (22)	61 (27)	62 (29)	0.96
	Final	59 (27)	47 (6)	58 (29)	62 (21)	0.44
Pain Relief [VAS, 0–100 mm, mean (SD)]	*Basal*	37 (29)	28 (31)	36 (30)	42 (31)	0.62
	Final	40 (28)	57 (21)	41 (30)	39 (22)	0.53
Quality of life [EQ, 0–100 mm, mean (SD)]	*Basal*	45 (24)	38 (26)	46 (25)	46 (21)	0.73
	Final	43 (22)	52 (8)	43 (23)	36 (12)	0.46
Opioid Withdrawal [OWS, 0–96 score, mean (SD)]	*Basal*	32 (19)	35 (25)	32 (18)	33 (29)	0.91
	Final	32 (20)	10 (0)	30 (20)	**46 (10)**[+]	**0.03**
Global Functionality [GAF, 0–100 score, mean (SD)]	*Basal*	71 (15)	74 (17)	70 (14)	80 (21)	0.48
	Final	69 (16)	90 (0)	69 (16)	69 (13)	0.40
Use of non-opioid adjuvants (%)						
Neuromodulators	*Basal*	48	50	52	**0**[+]	0.05
	Final	49	40	49	**11**[+]	0.09
Duloxetine	*Basal*	18	33	22	17	0.53
	Final	23	20	25	11	0.91
NSAIDs	*Basal*	8	0	7	0	0.63
	Final	5	0	4	0	0.52
Simple analgesics	*Basal*	25	17	27	33	0.80
	Final	13	40	12	0	0.09
Benzodiazepines	*Basal*	36	17	40	33	0.52
	Final	37	20	38	22	0.85

[†]Comparisons between PM, vs. EM, vs. UM, were performed using ANOVA, or Kruskal-Wallis test for continuous parametric and non-parametric data, respectively and Chi-square test for categorical data.

+*$p < 0.05$, **$p < 0.01$ basal vs. final (lowest value in bold) using *t*-test or U Mann-Whitney test for parametric and non-parametric data, respectively$p < 0.05$ UM vs. PM/EM (UM, value in bold and shaded in grey) using *t*-test or Fisher's exact test for continuous or categorical data, respectively. PM, poor metabolizer; EM, extensive metabolizer; UM, ultrarapid metabolizer; MEDD, morphine equivalent daily dose; CYP-Opioids, Opioids subject to metabolism by CYP2D6; VAS, visual analogue scale; EQ, VAS, EuroQol Scale (0–100 mm); OWS, opiate withdrawal scale; GAF, global assessment of functioning; IQR, interquartile range, expressed in parenthesis as P25 and P75.

no differences between CYP2D6 phenotypes and consumed MEDD were observed. What's more, CYP2D6-UMs presented a lower rate of neuromodulators use in comparison to the other phenotypes in both basal and final visits (0% vs. 51%, $p = 0.03$ and 11% vs. 49%, $p < 0.04$, respectively) with no differences between sex or time period.

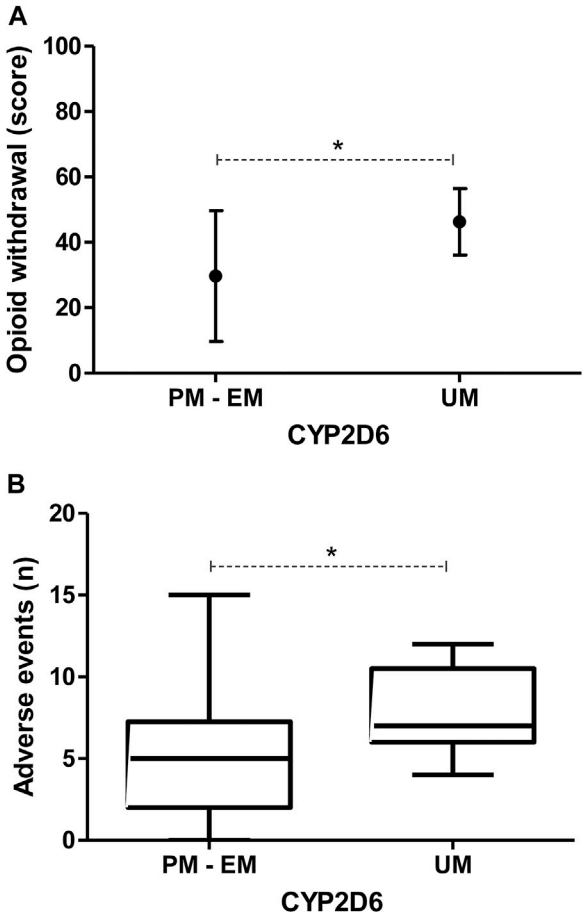

FIGURE 2
Final **(A)** opioid withdrawal scores (mean ± SD) and **(B)** total number of adverse events (boxplots) by *CYP2D6* metabolizer phenotype (PM, Poor Metabolizer; EM, Extensive Metabolizer; UM, Ultrarapid Metabolizer), showing a significant increase of both variables in UM. *p*-values <0.050 are represented with an asterisk.

3.3 Opioid deprescription outcomes and *CYP2D6* phenotype

At final visit, even though a significant reduction in MEDD and opioid use was reached, most of the clinical outcomes remained stable without any significant changed after opioid deprescription or cessation. Only men showed a non-significant reduction of quality of life [basal vs. final, 49 (24) vs. 38 (23) mm, $p = 0.05$] while women remained stable [43 (24) vs. 46 (21) mm, $p = 0.43$].

Related to CYP2D6, UMs subjects (Figure 2A) showed a 3-4-fold increase in opioid withdrawal (46 (10) in comparison to the other phenotypes [30 (20) OWS scores, $p = 0.01$] with a significant inverse correlation with levels of quality of life, both in males [$r = -0.572$ (−0.797 to −0.209), $p = 0.01$] and females [$r = -0.700$ (−0.841 to −0.470), $p < 0.001$] (Supplementary Figure S1) at final visit. What´s more, PMs final functionality clearly improves to a mean of 90 GAF scores, which means "absent or minimal symptoms, good functioning in all areas, interested and involved in a wide range of activities. socially effective, generally satisfied with life, no more than everyday problems or concerns." Whilst, UM decrease to 69 GAF scores, which means "some mild symptoms or difficulty in social, occupational, interpersonal relationships."

3.4 Adverse events

A median of 5 (2–8) AEs per patient were reported in cases, being the most prevalent dry mouth, sleep disturbance, constipation and nervousness (present in >40% of the patients), while controls showed a lower frequency of AEs [3 (1–6) AEs/patient, $p = 0.01$] (Table 1). Cases included in 2016–2019 showed a significant lower frequency of AEs [6 (4–9) vs. 2 (0–5), $p < 0.001$] compared to those included earlier (2013–2015) (Supplementary Table S1). Furthermore, a total of 13% of the cases presented some suspected ADR (ratio 60 AEs: 1 ADR) during the deprescription, mainly psychiatric or reproductive system's disorders.

Data related to AEs by CYP2D6 metabolic phenotype are shown in Table 3. Here, UMs showed a significantly higher mean of 7 (6–11) AEs/patient in comparison to the others phenotypes [5 (2–7) AEs/patient, $p = 0.02$], with higher frequencies of headache (100% vs. 33%, $p = 0.01$), edema (50% vs. 9%, $p = 0.02$), dry mouth (100% vs. 53%, $p = 0.03$) and nervousness (86% vs. 38%, $p = 0.04$) (Figure 2B and Supplementary Figure S2A). In accordance, UMs showed higher gastrointestinal (PM: 0 vs. EM: 71 vs. UM: 100, $p = 0.01$) and general (0% vs. 9% vs. 50%, $p = 0.01$) systems' disorders. No gene-sex interactions by regression model were found in those variables where CYP2D6 metabolic phenotypes showed differences (data not shown).

Related to sex, women reported a higher frequency of edema (15% vs. 0%, $p = 0.05$), dry mouth (63% vs. 33%, $p = 0.02$) and nervousness (50% vs. 22%, $p = 0.029$). Meanwhile, men retained sexual impotence issues at a significantly higher rate than females (25% vs. 4%, $p = 0.01$) mostly due to erectile dysfunction (Supplementary Figure S2B). What´s more, ADRs notified were three times higher in men than in women (23% vs. 7%, $p = 0.02$).

4 Discussion

Ambulatory opioid deprescription was effective in 76% of participants, where 42% ceased their opioid use. Here, CYP2D6-UMs showed the worst tolerability and high quality of life impact. Different frequencies of adverse events between sexes were reported that together with age and opioid dose could contribute to opioid dependence vulnerability.

This article also identifies priorities for monitoring younger, higher MEDD consumers with low tolerability CNCP patients who showed any misuse behavior. Current recommendations warn about a significant increase in OUD risk when the MEDD exceeds 90 mg/day (Busse et al., 2017; Webster, 2017). In our cases, a younger age and a higher median MEDD were found to be potential risk factors. Once OUD is detected, individualized decreasing dose regimen and/or opioid discontinuing is proposed based on clinical guidelines, which prevents the onset of withdrawal signs and symptoms (Nafziger and Barkin, 2018), as

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TABLE 3 Adverse events frequency at basal and final visit and analysis by metabolic phenotype.

Adverse events (%)	Visit	*CYP2D6* phenotype			*p*-value[†]
		PM (*n* = 7, 6%)	EM (*n* = 98, 85%)	UM (*n* = 10, 9%)	
Total (Median (IQR))	*Basal*	4 (2–10)	6 (3–8)	7 (3–14)	0.57
	Final	2 (0–3)	5 (2–8)	**7 (6–11)**[+]	**0.02**
Dry mouth	*Basal*	40	57	71	0.55
	Final	0	55	**100**[+]	**0.01**
Sleep disturbing	*Basal*	40	52	71	0.52
	Final	33	55	71	0.52
Constipation	*Basal*	40	45	57	0.81
	Final	0	48	43	0.26
Nervousness	*Basal*	40	42	57	0.73
	Final	0	40	**86**	**0.02**
Dizziness	*Basal*	40	**43**[*]	43	0.99
	Final	0	23	43	0.32
Headache	*Basal*	60	26	57	0.09
	Final	33	33	**100**[+]	**0.01**
Depression	*Basal*	40	34	43	0.88
	Final	0	39	75	0.05
Drowsiness	*Basal*	40	31	29	0.91
	Final	33	34	57	0.49
Weight change	*Basal*	0	35	43	0.24
	Final	0	28	17	0.49
Dry skin	*Basal*	20	31	43	0.70
	Final	0	38	67	0.14
Nausea	*Basal*	40	26	14	0.60
	Final	0	18	29	0.57
Itchy	*Basal*	40	25	43	0.49
	Final	0	27	43	0.37
Lack of appetite	*Basal*	20	28	43	0.65
	Final	0	23	57	0.09
Loss of libido	*Basal*	20	28	29	0.92
	Final	33	30	14	0.68
Vomiting	*Basal*	0	9	14	0.70
	Final	0	8	0	0.69
Edema	*Basal*	0	11	14	0.70
	Final	0	9	**50**	**0.01**
Skin redness	*Basal*	20	8	14	0.58
	Final	0	9	33	0.16

(Continued on following page)

TABLE 3 (*Continued*) Adverse events frequency at basal and final visit and analysis by metabolic phenotype.

Adverse events (%)	Visit	*CYP2D6* phenotype			*p*-value[†]
		PM (*n* = 7, 6%)	EM (*n* = 98, 85%)	UM (*n* = 10, 9%)	
Sexual dysfunction	*Basal*	0	9	0	0.54
	Final	0	14	0	0.49

[†]Comparisons between PM, vs. EM, vs. UM, for each visit were performed using ANOVA, or Kruskal-Wallis test for parametric and non-parametric data, respectively (significant $p < 0.05$ and highest value in bold). Multiple testing was adjusted with Bonferroni Correction where a *p*-value<0.017 was significant (+ highest value in bold and shaded in grey).
*$p < 0.05$ in basal vs. final (highest value in bold) using Fisher's exact test. PM, poor metabolizer; EM, extensive metabolizer; UM, Ultrarapid Metabolizer. IQR, interquartile range, expressed in parenthesis as P25 and P75.

happened in our case. Additionally, our data demonstrates that UM phenotypes showed 3–4 times increased opioid withdrawal and higher AEs numbers that could be crucial at an early OUD stage (Planelles et al., 2019) or increasing the risk of life-threatening reactions compared to regular metabolizers (Haufroid and Hantson, 2015). In our setting, 42% completed the program without opioid prescription. Here, adherence monitored by qualitative urine drug testing and/or gas chromatography mass spectrometry as confirmatory quantitative testing could be considered (Nafziger and Barkin, 2018).

The study provides clear directions that would lead to changes in clinical practice. As a primary hypothesis, it was considered that CYP2D6-UM phenotypes patients with an OUD would show a different clinical outcome pattern when deprescribing, mainly due to a worse safety profile. The potential benefits of using CYP2D6 phenotype could be especially relevant in southern European and Northern African populations that have higher proportions of UM (Kirchheiner et al., 2008). In these situations, when PM or UM are detected, it is important to consider using different analgesic drugs, such as those which are metabolized through a phase II metabolic pathway, in order to avoid a possible therapeutic failure. Here, oxymorphone immediate- and/or sustained-release formulations could be considered in countries where they are available. For its part, tapentadol, while being residually metabolized to inactive hydroxytapentadol (2%) by CYP2D6, it is largely glucoronidated via phase II and interindividual CYP2D6-related variability in the analgesic response is not expected (Barbosa et al., 2016), which makes tapentadol an alternative to consider.

This study aims to demonstrate the clinical interest of genotyping when deprescribing in order to identify patients at risk of insufficient analgesia or adverse events. In this way, there is also a need to carry out studies that analyze the cost-effectiveness of genetic testing when genotyping is included in these procedures. Along with this, it is important the need to develop clinical guidelines as a vehicle to assist the providers of opioids, in order to detect a potential issue not only with *CYP2D6,* but also with other P450 enzymes (*1A2, 2C9, 2C19* or *B6*).

Also, the need to implement pain research with a sex perspective is necessary to understand interindividual variability in terms of safety. Still, the remarkable female predominance in our study merits further attention. Nearly two thirds of our patients were adult women, given that female predominance in our CNCP population has been previously highlighted (Planelles et al., 2020). Furthermore, data showed that females communicated more AEs related to nervous, gastrointestinal and general systems, and less related to the sexual sphere in comparison to men, being third-less frequent ADRs in females (Muriel et al., 2019). Even more, surprisingly, men expressed a lower quality of life after opioid deprescription, while those of the women remained stable after deprescription. These different trends of impact related to the complex interdependence between biological sex and gender need to be elucidated (Becker and Chartoff, 2018; Rogers et al., 2020) because other factors (stress, depression, anxiety, responses to pain related to avoidance, coping) can have a greater impact on disability and quality of life, than on pain, *per se* (Sinha, 2008; Goodyear et al., 2018).

Some limitations should be taken under consideration. First, a convenience sample of patients attending a single pain clinic was established, along with this, a power analysis was not performed in order to know the best scenario to detect differences between groups. Furthermore, the total number of extreme phenotype subjects studied was relatively small. All this can compromise the power of statistical analyses, which may have made it difficult to detect significant differences between groups. Second, an 80% of UM were females, it would be difficult to assess the effect of CYP2D6 on the observed clinical outcome. Even more, drug inhibition or induction effects on CYP2D6 should be deeply analyzed (Kosten and Baxter, 2019), because it can condition the level of MEDD reduction (Smith et al., 2019). Furthermore, pharmacological data was obtained from EHRs and potential mismatches between the patients' intake and prescribed doses could exist. Other drugs or interventions less commonly used in our setting such as tricyclic antidepressant, cannabinoid or nerves block should be explored in further analyses. Third, with basal and final visit data available, it is preferable to analyze the repeatedly measured data together instead of separate statistical tests, but the low frequency of extreme phenotype subjects limited its execution. Finally, since the inclusion period was long and substantial changes could have occurred, such as increased physician experience in deprescribing and/or new indications for available drugs, among others, subjects included in 2013–2015 and those in 2016–2019 were compared to determine if deprescription outcomes changed over time. Here, statistical significance was not reach for deprescription response, but lower MEDD (51% of the subjects ended with no opioids) combined with a welcome lower frequency of AEs were observed while clinical variables remained stable, strongly suggesting an improvement in the deprescription procedure over time.

In conclusion, CYP2D6 metabolizer phenotypes may contribute to differential and improved opioid deprescription in CNCP. Sex

may play a relevant role in the tolerability when deprescribing. Further studies considering these potential genetics, as well as sex/gender differences could help to understand the interindividual variability in real-world patients.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of The General University Hospital of Alicante. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JM, JB, CM, and AP conceived and designed the study. JM, JB, GD, CC, and PB conducted most of the experiments. JM, JB, and ME carried out data analysis and wrote the manuscript. GD and CC participated in collecting data and helped to draft the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1200430/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Opioid withdrawal and quality of life inverse correlation after deprescription programme in OUD males and females.

SUPPLEMENTARY FIGURE S2
Percentage of Adverse Events at final visit classified by: **(A)** CYP2D6 metabolic phenotype (PM, Poor Metabolizer; EM, Extensive Metabolizer; UM, Ultrarapid Metabolizer); **(B)** Sex-differences (F, female, M, male). *p*-values <0.050 are represented with an asterisk.

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The pharmacogenomics of carbamazepine-induced cutaneous adverse drug reaction in the South of Vietnam

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Background: The relationship between *HLA-B*15:02* and Severe Cutaneous Adverse Reactions was rigorously examined in Japanese, Han Chinese, Thais, and Caucasians. However, the number of studies about this topic in Vietnamese population is still limited and mostly focuses on the North of Vietnam.

Objective: This study aims to clarify the genetic culprit of SCARs in Vietnamese population, particularly in the South of Vietnam, and to validate our result by a meta-analysis about this topic in Vietnamese.

Method: A retrospective case-control study with 37 patients treated with carbamazepine monotherapy. Statistical calculation and meta-analysis were performed by R software.

Result: *HLA-B*15:02* increases the risk of SJS 12.5 times higher in CBZ-treated patients (p-value = 0.017). However, this allele has no impact on MCARs (Mild Cutaneous Adverse Reactions) of CBZ. The number needed to test and the number needed to genotype is two and nine patients respectively.

Conclusion: This study recommends more investigations about the cost-effectiveness of this test to accelerate the protection of Southern Vietnamese from SCARs.

KEYWORDS

pharmacogenomics, Vietnam, HLA-B*15:02, SJS, MCARs, SCARs, epileptic, carbamazepine

1 Introduction

George Snell (Snell, 1981), a Nobel laureate, discovered the Major Histocompatibility Complex (MCH) in 1948. Ten years later, the first Human Leucocyte Antigen (HLA) was detected (Thorsby, 2009). Since then, HLA genes have been gradually unveiled to be one of the most sophisticated genes with over 35,000 alleles confirmed by the time of this study (Barker et al., 2023). Different alleles render different amino acids in MCH molecules, where the antigen presentation happens in a manner of specificity. MCH contains two major classes designated as HLA class I and HLA class II, which are respectively responsible for the

endogenous and exogenous pathways. Moreover, the polymorphism of HLA genes plays a crucial role in the development of Steven-Johnson (SJS) and Toxic epidermal necrolysis (TEN).

The diversity of genes encrypted for HLA protein is not only reflected in one single ethnicity, where this diversity allows a wide range of antigens to be recognized and responded to, but also reflected in the differences between ethnicities. Most of the genetic causes of Carbamazepine-induced SCARs were investigated in developed Asian countries, such as Han Chinese (Wang et al., 2011; Zhang et al., 2011), Thais (Tassaneeyakul et al., 2010; Sukasem et al., 2018), and Taiwanese (Chen et al., 2011). However, Southeast Asian populations have received less attention, especially in the South of Vietnam where the flow of immigration created an admixture of crowded population. The South of Vietnam is the home of around 40 million people (2023) with diverse ethnicities such as Kinh Vietnamese, Khmer Krom, Cham Vietnamese, etc. In Caucasians, Japanese, and Koreans, even though a myriad of meticulous research has been conducted about *HLA-B*1502*, this allele is reported to be rare and not associated with statistically significant risk in these populations. In contrast, the *HLA-B*15:02* allele was believed to have the highest allele frequency and fatal risk in the general population of Southeast Asia (Moutaouakkil et al., 2019).

Indeed, the relationship between *HLA-*B-15:02* and SCARs was rigorously examined in Japanese, Han Chinese, Thais, and Caucasians. However, the number of studies about this topic in Vietnamese population is still limited and mostly focuses on the North of Vietnam (Van Nguyen et al., 2015; Van Nguyen et al., 2022). Given that Vietnam is a highly populated and racially diverse country, which can create genetic heterogeneity, more research is needed to fully understand the genetic predisposition of Vietnamese, especially those in the south of Vietnam. Unfortunately, as an economically developing area, Vietnam faces tremendous financial barriers in scientific research, which may result in the most vulnerable population possibly receiving the least pre-emptive protection.

*HLA-B*15:02* is not only important in the treatment of Carbamazepine, but also in its structural analog, Oxcarbazepine (Phillips et al., 2018). The cross-reactivity of *HLA-B*1502* contributes to the improvement in cost-effectiveness, making this allele a worthy investment for personalized therapy.

In this study, we aimed to clarify the genetic culprit of SCARs in the Vietnamese population, particularly in the South of Vietnam. We also compare the associated risk and genetic patterns in the population between the south and the north of Vietnam or other countries.

2 Methods

2.1 Study design and participants

This is a case-control study with the control group defined by patients tolerant with CBZ. The study was conducted in the Department of Neurology at NDGD Hospital from 1 January 2019 to 31 December 2020. The analysis included a total of 7 cases of CBZ-induced SJS, 12 cases of CBZ-induced MCARs, and 18 cases of CBZ-tolerant control as shown in Figure 1.

All patients were recruited retrospectively with the definitions based on the information written in the medical records. The definition of CBZ tolerant is the patients who administered CBZ and the attending physician did not write any diagnosis of SJS or other skin manifestations of allergy. The definition of CBZ-induced SJS is the patients who administered CBZ and the attending physician wrote the diagnosis of SJS. The definition of CBZ-induced MCARs is the patients who administered CBZ and the attending physician wrote the diagnosis of itching skin, hypersensitivity reaction, CBZ allergy, anaphylaxis level 1, skin reaction, or symptomatic allergy. The patients who administered both CBZ and PHT were excluded from this study, only epileptic patients treated with monotherapy CBZ were included. Patients without information of genotype were also excluded from this study. The diagnosis of SJS/TEN was performed by certified dermatologists in accordance with the hospital's guidelines, which included the following criteria: 1) Onset of symptoms within 2 months of initiating CBZ. 2) Presence of a rash affecting the face, upper body, limbs, or spreading extensively across the entire body. 3) Mucosal damage observed in at least two natural cavities, such as the eyes, nose, mouth, vagina, or anus. 4) Skin detachment (10% for SJS, 30% for TEN) or the presence of Nikolsky's sign.

2.2 Genotyping methods

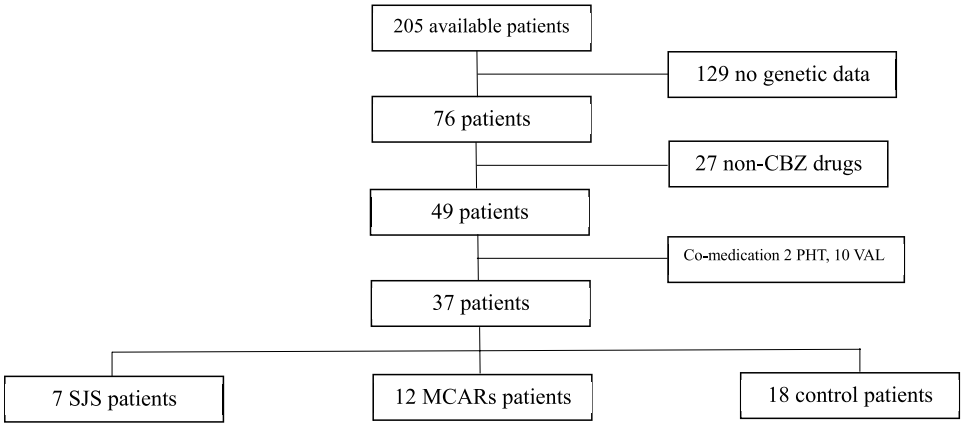
Within 76 patients with available DNA, 30 DNA samples were sent to the genotyping service at the laboratory of Pharmacogenomic and Personalize Medicine (PPM) of Ramathibodi Hospital Mahidol University, Bangkok, Thailand, 46 DNA samples were sent to the genotyping service at University Medical Center (UMC), University of Medicine and Pharmacy at Ho Chi Minh city. The genotyping method used at PPM was Luminex™ flow cytometry. In brief, the sample DNA binds complementarily to a panel of probes, which was designed with known nucleic sequences. The fluorescence detection technology was used to identify successful binding complexes, and hence, identify the sequence and genotype of the sample. The genotyping method used at UMC was real-time Polymerase Chain Reaction (real-time PCR), using Tagman™ genotyping assay.

2.3 Statistical analysis

All statistical analyses were done by R software version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Student's t-test was used to compare the differences between 2 independent groups. Chi-squared tests were used to compare the ratio of males and females between 2 groups; Fisher's exact test was employed to compare the risk of alleles between the cases and the controls.

2.4 Meta-analysis

This analysis aimed to compare our conclusion with the Vietnamese population in the both the North and the South by evaluating the impact of *HLA-B*15:02* in Vietnamese epileptic patients treated with CBZ, utilizing various databases such as Pubmed, Google Scholar, Cochrane Library, and ScienceDirect



CBZ: Carbamazepine; Non-CBZ: Medication other than carbamazepine; PHT: Phenytoine; VAL: Valproic acid.
SJS: Stevens-Johnson syndrome; MCARs: Mild cutaneous adverse drug reaction;

FIGURE 1
Recruitment process of epileptic patients.

(Figure 2). The keywords used were "*HLA-B*15:02* Vietnamese", "*HLA-B*15:02* Vietnam", "SJS", "Steven-Johnson Syndrome". To select the suitable studies, the selection criteria were: 1) Containing the case-control analysis of *HLA-B*15:02* and SJS, 2) Targeting Vietnamese epileptic patients, 3) Including the clear definitions of case and control with corresponding numbers, and the exclusion criteria were: 1) Duplicating studies, 2) Researching about the development of genotyping methods in Vietnam, 3) Being a case report or cross-sectional study. Data analysis and visualization were performed on Rstudio, using random effect with a *p*-value lower than 0.05 is considered statistically significant, and with I^2 higher than 50% was defined as heterogeneous. The estimation method used for the random effect is restricted maximum likelihood.

2.5 Ethics approval

This study was approved by the Ethics Committee of NDGD Hospital, Ho Chi Minh City, Vietnam, under approval number 23-2015/CN-HĐĐĐ, on 13 October 2015. As we used only retrospective data from health records while also maintaining patient confidentiality, no informed consent was required for this study.

3 Result

3.1 Genetic and demographic information of patients

Table 1 describes the characteristic of the participants. The age of patients was widely distributed from 25 to 85 years old. The separation of gender was fairly equal between males (21 patients) and females (16 patients) in total participants. However, there was a higher proportion of males in the SJS group. The percentage of males respectively was 50% and 55.6% in the group of CBZ-induced MCARs and CBZ-tolerant control. All three groups contained patients with chronic diseases, such as hypertension or dyslipidemia. Nearly 30% of epileptic patients had a medical history of cerebral infarction or brain hemorrhage.

Among twelve carbamazepine-induced MCARs patients, five patients were diagnosed with itching skin and hypersensitivity reactions, six patients were diagnosed with symptomatic allergy and skin reactions of allergy, and one patient was diagnosed with anaphylaxis level 1 due to CBZ allergy. It is important to note that none of the twelve patients exhibited symptoms specific enough to be classified as SJS/TEN.

3.2 The frequencies of *HLA-B* alleles detected in Southern Vietnamese

Table 2 shows the HLA allele polymorphism in the epileptic southern population of Vietnam, where a total of 22 alleles were observed. The most popular *HLA-B* alleles are *15:02* (20%), *38:02* (10%), and *46:01* (10%). There is a 5% of frequency in each of the following alleles: 7:05; 18:01, 37:01, 57:01. The rest 15 alleles account for around 1.7%–6.7% of the whole population.

3.3 The increased risk patients carrying *HLA-B*15:02* in Southern Vietnamese

Table 3 illustrates that in the Southern Vietnamese population, the HLA-B*15:02 allele is a statistically significant risk factor for SJS (p = 0.017), but not for MCARs (p = 0.660). The odd ratio of the *HLA-B*15:02* allele is 12.5. In other words, the carriers of this allele have 12.5 times higher risk than non-carriers, in terms of SJS. The sensitivity and specificity are 71.4% and 83.3% respectively, meaning that the hospital's protocol can correctly identify 71.4% of SJS patients, and correctly identify 83.3% of non-SJS patients.

TABLE 1 Demographic data of recruited epileptic patients.

	CBZ-induced SJS (n = 7)	CBZ-induced MCARs (n = 12)	CBZ-tolerant control (n = 18)	*p* values ∓
Gender (number; percent)				
Male	5 (71%)	6 (50%)	10 (55.6%)	0.785; 1.000
Female	2 (29%)	6 (50%)	8 (44.4%)	
Age (years)				
Min	30	25	31	0.4813; 0.3227
Average	51	49.8	56.2	
Max	83	85	81	
Group of age				
25–45	2	6	6	
46–65	4	3	9	
66–87	1	3	3	
Height (cm)				
Min	150	148	150	0.5821; 0.3097
Average	164.6	159.6	162.6	
Max	173	172	176	
Weight (kg)				
Min	51	43	47	0.5954; 0.4199
Average	62.3	57	59.7	
Max	86	73	80	
Diagnosis other than epilepsy				
Hypertension	1	1	7	
Dyslipidemia	1	2	6	
Implication of cerebral infarction	1	2	5	
Implication of brain hemorrhage	2	0	1	
Cerebral vascular insufficiency	0	0	5	
Liver disease	2	1	0	
Osteoporosis	1	0	1	

SJS: Stevens-Johnson syndrome; ∓: The first *p*-value is between CBZ-induced SJS, and tolerant control. The second *p*-value is between CBZ-induced MCARs, and tolerant control. *p* values were received from the Student's t-test (except for gender variables, which used the Chi-squared test). A *p*-value lower than 0.05 are considered statistically significant (two-sided); cm: centimeter; kg: kilogram.

TABLE 2 The frequencies of detected *HLA-B* alleles in the population of Southern Vietnamese.

Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
7:05	0.05	15:25	0.033	38:02	0.1	51:01	0.017
13:01	0.017	18:01	0.05	39:01	0.017	53:04	0.017
15:01	0.017	27:04	0.033	40:01	0.067	57:01	0.05
15:02	0.2	27:06	0.033	44:03	0.017	58:01	0.033
15:08	0.017	35:05	0.033	46:01	0.1		
15:12	0.033	37:01	0.05	50:01	0.017		

HLA-B: Human leucocyte antigen class 1 B; Freq: Frequency.

Besides, the PPV and NPV are 62.5% and 88.2% respectively. These two parameters are interpreted in the context that the results of HLA-B*15:02 are available. In these cases, if the result of a patient is positive, the possibility for this patient to have SJS is 62.5%, and if the result of a patient is negative, the possibility for this patient to not have SJS is 88.3%. Moreover, the NNT and NNG are respectively 2 and 9, which confirms that to protect 1 patient from SJS, the intervention of replacing CBZ with an alternative drug has to be done in 2 patients, or the genetic test has to be done in 9 patients.

Out of the twelve cases of allopurinol-induced MCARs, three patients tested positive for *HLA-B*15:02*. While the strength of evidence is not large enough to be statistically significant, it is worth noting that the rate of *HLA-B*15:02* in the MCARs group is higher compared to the group of tolerant controls.

3.4 Meta-analysis

A meta-analysis was performed about the risk of *HLA-B*15:02* and SCARs in Vietnamese with a total of 6 studies (Van Nguyen

TABLE 3 The association and odd ratio of *HLA-B* alleles.

HLA-B	Case	Tolerant control	OR (95% CI)	*p* values	Sens (%)	Spec (%)	PPV (%)	NPV (%)	NNT	NNG
SJS										
15:02	5/2	3/15	12.50 (1.60, 97.65)	0.017	71.4	83.3	62.5	88.2	2	9
MCARs										
15:02	3/9	3/15	1.67 (0.28, 10.09)	0.660	25	83.3	50	62.5	8	40

SJS: Stevens-Johnson syndrome; MCARs: Mild Cutaneous Adverse Reactions; OR: odd ratios; 95% CI: 95% confident interval; *p* values were calculated by Fisher exact test with the significance threshold of 0.05; Sens: Sensitivity; Spec: Specificity; PPV: positive predictive value; NPV: negative predictive value; NNT: number needed to treat; NNG: number needed to genotype.

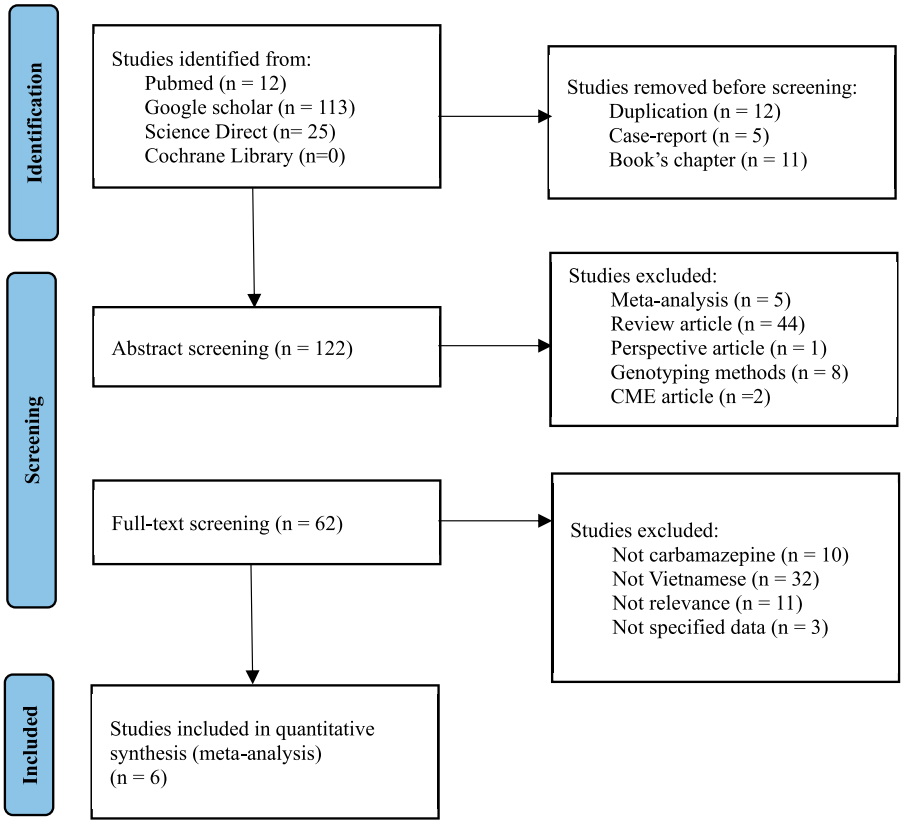


FIGURE 2
PRISMA flowchart for identification of eligible studies.

et al., 2015; Van Nguyen et al., 2017; Huyen et al., 2020; van Nguyen et al., 2021; Chu, 2022; Bui et al., 2023). All these 6 studies were conducted and recruited patients exclusively in the northern region of Vietnam, specifically at Bach Mai Hospital, Tam Anh Hospital (Ha Noi branch), and the National Hospital of Dermatology and Venerology. Surprisingly, no eligible studies conducted in the southern region of Vietnam were identified or included in this meta-analysis (Supplementary Table S1). Interestingly, a recent study by Bui TP et al. (Bui et al., 2023) reported a negative association between *HLA-*B15:02* and SCARs in Vietnamese in January 2023. However, our analysis using pooled data from all six studies showed a strong positive association between *HLA-B*15:02* and SCARs, as determined by both common effect and random effects models (Figure 3). Carriers of *HLA-B*15:02* had 12.82 times higher odds of developing SCARs compared to non-carriers (p-value <0.01). The odd ratio calculated from previous studies in the meta-analysis was consistent with the odd ratio in the current study.

4 Discussion

This is the first case-control study to investigate the pharmacogenomics of CBZ-induced SJS in Southern Vietnam. Based on the evidence that the pharmacogenomic test can protect one case of SJS with every two interventions or every

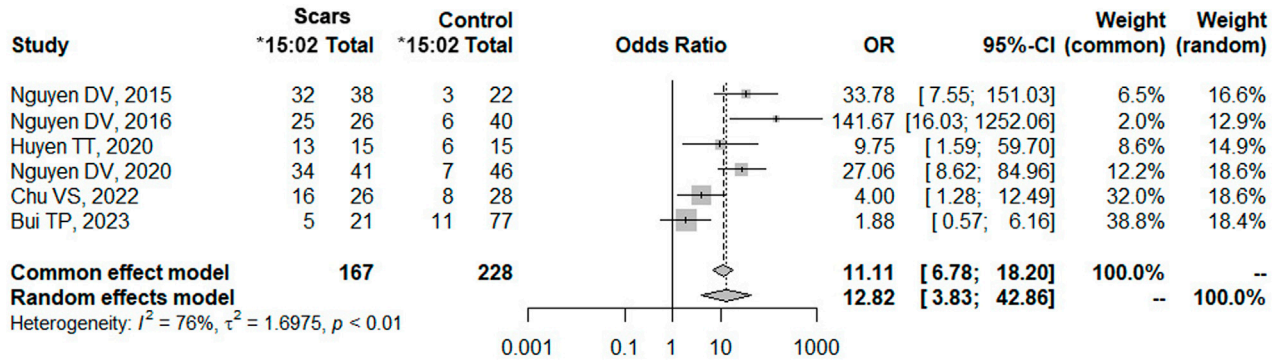


FIGURE 3
Forest plot of the association between *HLA-B*15:02* and SCARs in Vietnamese.

nine genetic tests, we recommend implementing this test in the population of Southern Vietnamese.

In the population of Southern Vietnamese, our study reported a higher frequency of the *HLA-B*15:02* allele, 20% compared to 11.88% in a study of the general population of Southern Vietnamese by Do MD et al. (Do et al., 2020). In the population of Northern Vietnamese, the frequency of *HLA-B*15:02* in epileptic patients varies between studies 13.5% (Van Nguyen et al., 2015), 15.2% (van Nguyen et al., 2021), 17.7% (Bui et al., 2023), and 41.7% (Huyen et al., 2020). The possible explanation may be due to the different regions or the small sample size, which can only reflect a part of the whole population. Interestingly enough, Que TN et al. (Que et al., 2022) reported a frequency of 15.11% of *HLA-B*15:02* with a sample size of 3750 participants, who were recruited in Northern and North-central Vietnam (Que et al., 2022). Besides, we found the frequencies of *HLA-B*38:02* and *46:01 to be equally 10%. Comparatively, Do MD et al. (Do et al., 2020) reported these two alleles to be 7.92% and 9.41% respectively. Que TN et al. (Que et al., 2022) also reported these two alleles to be 7.29% and 10.7% respectively.

Regarding global populations, Southeast Asians are regarded as having the highest frequency of *HLA-B*15:02* in the world (Moutaouakkil et al., 2019; Van Nguyen et al., 2019). Indeed, numerous studies published the frequency of *HLA-B*15:02* in their own country, such as 15.11% in Thailand (Zhang et al., 2011), 32.8% in Indonesia (Khosama, 2017), 8.3% in Malaysia (Chang et al., 2011), and 5.7% in Singapore (Middleton et al., 2004). Moving toward northeast Asia, this frequency gradually decreases, such as 7.3% in Southern Chinese (Trachtenberg et al., 2007), 1.9% in Northern Chinese (Hong et al., 2005), 7.7% in Taiwanese (Chen et al., 2011), 10.2% in Hongkong (Middleton et al., 2004), 1.7% in Japanese (Ikeda et al., 2010) and 0.4% in South Koreans (Kim et al., 2011). In contrast, *HLA-B*15:02* is seemingly absent in Caucasians, such as French (Gourraud et al., 2015), and the United States (Maiers et al., 2007).

The clinical implication of *HLA-B-*15:02* is specifically related to SJS and not MCARs, even though both are the cutaneous manifestations of allergy. This observation can be explained by recent immunologic findings that the CBZ hyperactivity reaction is not solely dependent on the specificity of HLA, but also the specificity of the T-cells and their receptors (Ko et al., 2011; Ko and Chen, 2012). This evidence also explains why three patients in our study tested positive for *HLA-B*15:02* but did not develop SJS.

Pharmacogenomics and personalized medicine are well-known to be ethnicity-specific. Even though in the same country, the genetic diversity could be significant and should not be ignored. This is especially true in the case of Vietnam, with a population of 100 million or 54 ethnicities, concentrated mainly in two metropolitan and healthcare centers of the north and the south. The absence of medical evidence in an ethnic group can impede the clinical implementation of a beneficial intervention. This is not only a medical issue, but also an ethical problem to have an equitable healthcare system (Patrinos et al., 2023). Besides the genetic differences, the socioeconomic and academic differences between regions also play an important role in pharmacogenomic research and implementation to ensure cost-effectiveness in resource-limited hospitals (Nagar et al., 2019).

Several limitations need to be rectified by future research. Firstly, the patients were genotyped by two genotyping methods, which may have increased the potential for bias. The decision to employ these methods was necessitated by the challenging circumstances presented by the COVID-19 pandemic. As a result, the *HLA-B*15:02* genotyping had to be conducted at a different hospital. Therefore, the better choice would be to use only one genotyping method. Secondly, the sample size is relatively small, larger studies should be conducted to clarify the finding in this study more thoroughly, especially for the outcome of MCARs in Southern Vietnamese. Thirdly, the exclusion of patients without genotyping results is another limitation, and future endeavors with more financial support should aim to include more patients to obtain a more comprehensive understanding of the real-world population.

In conclusion, this study confirms the association between CBZ-induced SJS and *HLA-B*15:02* in Vietnamese, particularly in the Southern population. Therefore, it is recommended to perform further studies about the cost-effectiveness of this test to accelerate the protection of Southern Vietnamese from SCARs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of NDGD Hospital, Ho Chi Minh City, Vietnam, under approval number 23-2015/CN-HĐĐĐ, on 13 October, 2015. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceived and designed the experiments: CS, HP; Collect demographic data, performed the experiment, and analyzed the data: QN, A-HN; Interpretated the finding: CS, HP, A-HN; Drafted the manuscript: A-HN; Adjusted the manuscript: HP, CS. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1217516/full#supplementary-material

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Lessons from clinical implementation of a preemptive pharmacogenetic panel as part of a testing pilot program with an employer-sponsored medical plan

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Introduction: This manuscript reports on a pilot program focused on implementing pharmacogenetic testing within the framework of an employer-sponsored medical plan at University of Florida (UF) Health. The aim was to understand the challenges associated with program implementation and to gather insights into patient attitudes towards PGx testing.

Methods: The pilot program adopted a partially preemptive approach, targeting patients on current prescriptions for medications with relevant gene-drug associations. Patients were contacted via phone or through the MyChart system and offered pharmacogenetic testing with no additional direct costs.

Results: Of 244 eligible patients, 110 agreed to participate. However, only 61 returned the mailed DNA collection kits. Among these, 89% had at least one potentially actionable genotype-based phenotype. Post-test follow-up revealed that while the majority viewed the process positively, 71% preferred a consultation with a pharmacogenetic specialist for better understanding of their results. Barriers to implementation ranged from fatigue with the healthcare system to a lack of understanding of the pharmacogenetic testing and concerns about privacy and potential misuse of genetic data.

Conclusion: The findings underscore the need for clearer patient education on pharmacogenetic results and suggest the importance of the role of pharmacogenetic-trained pharmacists in delivering this education. They also highlight issues with relying on incomplete or inaccurate medication lists in patients' electronic health record. The implementation revealed less obvious challenges, the understanding of which could be beneficial for the success of future preemptive pharmacogenetic implementation programs. The insights from the pilot program served to bridge the information gap between patients, providers, and pharmacogenetic -specialists, with the ultimate goal of improving patient care.

KEYWORDS

pharmacogenomics, implementation, pharmacogenetic panel, patient perspective, precision medicine, preemptive testing

Introduction

Precision medicine implementation is multifaceted, with pharmacogenetic (PGx) testing emerging as a prominent component. Over the past decade, the accessibility of PGx testing has markedly improved for patients and clinicians, driven by reduced testing costs, clinical guideline publications, and increased availability of commercial and institutional testing (Relling et al., 2020).

Despite these advancements, considerable challenges persist in delivering the benefits of PGx testing to patients. The cost of genotyping has decreased significantly over the past decade, yet a clinical PGx test may remain prohibitively expensive for many patients (Duarte et al., 2021). While insurance coverage of PGx testing has expanded, inconsistent reimbursement rates and level of coverage among payers may still serve as a relevant barrier to testing for some patients (Lemke et al., 2023). Furthermore, widespread implementation may be hampered by limited patient and provider education on PGx test utilization (Rahawi et al., 2020). Moreover, the interpretation and application of PGx test results demand expert clinical input to aid optimal clinical integration.

PGx testing is typically done reactively or preemptively. Reactive PGx testing is performed in response to initiating or planning to initiate a medication or after a patient experiences a suboptimal medication response suspected to be secondary to genetic variation (e.g., adverse event, treatment failure). Fully preemptive testing is conducted prior to the start of therapy, which may be days, months, or even years in advance of needing to use the genetic information. Preemptive testing is often done with multi-gene PGx panels, which provide lifelong results that inform prescribing decisions for a wide range of medications that may be used in the future (Greden et al., 2019). Panel-based PGx testing may also be implemented using a partially preemptive approach. With this approach, initial panel testing is reactive and performed in populations that meet specific inclusion criteria, such as patients taking a specific medication or those who may be at high risk for adverse events or treatment failure (Duarte et al., 2021). While only a subset of the genes included on a multigene panel may be necessary to address a patient's current needs, inclusion of additional genes can inform future prescribing.

It is estimated that 9 out of 10 patients have at least one actionable phenotype relevant to current or potential future therapies (Van Driest et al., 2014; Ji et al., 2016). Despite the potential for clinical utility, many patients are not undergoing testing for various reasons, including access. Implementation of panel-based PGx testing at the payor level may improve access for health plan members and reduce costs associated with adverse drug reactions and ineffective treatments. A recent example is the Teachers' Retirement System of the State of Kentucky, who, in partnership with Coriell Life Sciences and Know Your RX Coalition, provided PGx testing and pharmacist-led medication management services to over 5,000 of their Medicare eligible health plan members (Jarvis et al., 2022). The Teachers' Retirement System is a state-run pension program limited to retired Kentucky public school teachers and their spouses who are Medicare eligible. Decreased costs and utilization of acute care services were both observed among their members as a result of this partnership.

In an effort to find a model that could offer an accessible option for PGx testing to their qualifying patients, University of Florida (UF) Health partnered with GatorCare, a self-funded employer-sponsored medical and pharmacy benefit plan for employees and their families at UF and UF Health Systems. Establishing a sustainable model is crucial, and implementing it through a pilot period has proven to be a successful approach (Cicali et al., 2019; Cicali et al., 2023). We implemented a pilot where panel-based PGx testing was provided without direct costs to patients and buccal swab DNA collection kits were mailed to the participant's home. The overall purpose of this pilot was to develop a feasible method for providing partially preemptive PGx testing to UF Health patients during the coronavirus (COVID-19) pandemic. In order to improve PGx panel testing implementation models, both successes and challenges encountered are described. Additional implementation metrics were collected to gain an understanding of the real and perceived clinical usefulness of partial preemptive PGx panel testing.

Methods

Genotyping

PGx testing was performed with GatorPGx, a panel-based PGx test offered by UF Health's internal laboratory, which is a College of American Pathologists/Clinical Laboratory Improvement Amendments (CAP/CLIA) certified laboratory. The GatorPGx panel tests for 8 pharmacogenes (*CYP2C19, CYP2D6, CYP2C9, CYP3A5, SLCO1B1, CYP2C* cluster, *CYP4F2,* and *VKORC1*) using the QuantStudio 12K Flex Real Time PCR System (Applied Biosystems by Life Technologies) and Life technology TaqMan® SNP Genotyping Assays (Marrero et al., 2020). The GatorPGx panel has the capability to detect CYP2D6 copy number variations, however, it is not able to specify which allele nor how many copies are present. A full list of the individual star alleles and single nucleotide polymorphisms tested for can be found in Supplementary Figure S3. Phenotypes were derived based on guidance from The Clinical Pharmacogenetics Implementation Consortium (CPIC). Of note, previous activity score cutoffs for defining CYP2D6 metabolizer phenotypes are reported as these were used at the time of this implementation (i.e., activity score of 1.0 is defined as normal metabolizer). (Caudle et al., 2020).

Patient eligibility and enrollment

Published Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines and previously implemented gene-drug pairs at UF Health were used to curate a list of 27 medications that have clinically relevant pharmacogenetic association(s) with a gene included in the GatorPGx panel. For this paper, these 27 medications are referred to as "panel drugs; " a complete list of panel drugs can be found in Table 1.

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TABLE 1 Panel drugs (n = 27).

Pain	Relevant Gene(s)	Mental health	Relevant Gene(s)
Celecoxib, ibuprofen, flurbiprofen, meloxicam, piroxicam	CYP2C9	Atomoxetine, fluvoxamine, paroxetine	CYP2D6
Codeine, hydrocodone, tramadol	CYP2D6	Citalopram, escitalopram, sertraline	CYP2C19
GERD/H.Pylori	**Relevant Gene**	**Other**	**Relevant Gene(s)**
Dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole	CYP2C19	Tacrolimus	CYP3A5
		Tamoxifen	CYP2D6
Cardiovascular	**Relevant Gene(s)**		
Clopidogrel	CYP2C19	Phenytoin	CYP2C9
Simvastatin	SLCO1B1	Voriconazole	CYP2C19
Warfarin	CYP2C9, VKORC1 CYP2C cluster		

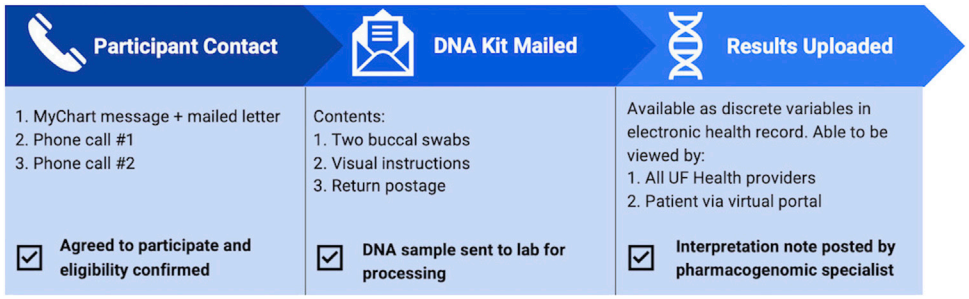

FIGURE 1
Flow of patient experience throughout the pilot program. Eligibility was determined based on review of electronic health record, then confirmed with the patient once contact was established. Upon accepting participation in the pilot, patients were mailed a DNA collection kit and once returned, pharmacogenetic results were uploaded to the electronic health record.

UF Health patients were able to participate in the pilot if they were: 18 years old and older, had active enrollment in a GatorCare benefit plan, an active prescription for a panel drug regardless of duration of therapy, and at least one outpatient visit at UF Health in the preceding 12 months. The pilot was registered with UF Health as a quality improvement project. Authors reviewed the electronic health record (EHR) to identify eligible patients and contacted relevant providers at various UF Health clinics to opt out if they did not want their patients to participate or be contacted by the pilot program. Participating UF Health providers included 19 family medicine providers, 13 gastroenterologists, and 3 internal medicine providers. Providers were also given the opportunity to self-identify patients who may benefit from testing and offer enrollment in person, so long as they fit eligibility criteria. If they desired to do so, instructions and materials for DNA sample collection were given to the respective provider. Patients were offered the opportunity to participate between October 2020 and March 2021. Eligible patients were contacted both through MyChart messages and mailed letters (Figure 1). The outreach information included educational PGx information and described the process of undergoing PGx testing through the pilot program (Supplementary Figures S1, S2). If there was no response to the message or mailed letter, two follow-up phone calls were performed, each at least 1 week apart. Once contact was established, medications and benefit plan enrollment were confirmed directly with the patient to verify eligibility for the pilot. If the patient declined participation, the reason was documented. If the patient did not respond after two letters and two follow-up phone calls, they were assumed to have declined participation.

Sample collection and results

If the patient agreed to participate in the pilot, a buccal DNA collection kit was mailed directly to the patient, which included a pre-paid mailing envelope to return the sample to the laboratory. If patient was identified and agreed to participate in person, the buccal sample was collected in clinic. After processing the samples and running the GatorPGx assay, the laboratory returned the genotypes and phenotypes to the EHR as discrete variables. Once available in the EHR, the pharmacy team was notified, UF Health providers were able to review the results in Epic, Best Practice Alerts (BPAs) were

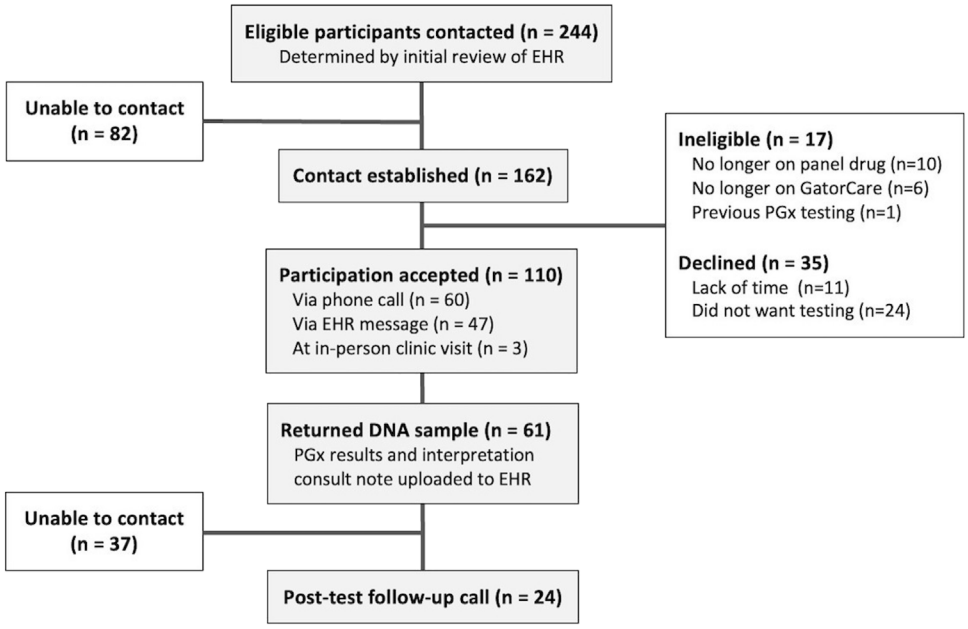

FIGURE 2
Patient participation and involvement. Eligibility criteria included actively prescribed a panel drug, currently a GatorCare benefit plan member, and having had at least one clinic visit within UF Health in the last year.

TABLE 2 Patient demographics.

Demographic	*n* (%) total *n* = 61
Age (years)	
Minimum	25
Mean (SD)	50.93 ± 10.21
Maximum	71
Sex	
Female	39 (63.9%)
Male	22 (36.1%)
Race	
White	53 (86.9%)
Asian	3 (4.9%)
Other	3 (4.9%)
Black or African American	2 (3.3%)
Ethnicity	
Not Hispanic or Latino	59 (96.7%)
Hispanic or Latino	1 (1.6%)
Unknown/Not Reported	1 (1.6%)

able to fire, and patients were able to review results via MyChart. Upon pharmacy notification that the results were returned, a pharmacogenetics-trained pharmacist reviewed the results within 24–48 h and wrote a consult note that included interpretations of the patient-specific genotype/phenotype and recommendations for current and future medications. The consult note was documented in the EHR and routed to the participant's provider via Epic in-basket message.

Post-test data analysis

Patients who completed PGx testing received a post-test follow-up phone call to inquire about overall satisfaction and perception of the pilot program, as well as to obtain perspectives on the clinical utility of testing. Post-test follow-up phone calls were attempted between September 2021 through March 2022 and occurred a minimum of 3 months after patients' PGx test results were resulted to the EHR. If contact was successful, patients were asked the following questions and asked for a verbal response: 1) *What is your overall opinion of the process? 2) Would you have preferred a follow-up appointment with a PGx specialist to explain the results? 3) Do you feel like these PGx results may have a potential impact on your care?* Any additional comments made by the patient relating to their experience or perspective on the program were documented. Data were analyzed descriptively (mean ± standard deviation or as frequencies), with pairwise comparisons performed using Student's t-test. Metrics collected to gain an understanding of the real and perceived clinical usefulness of partial preemptive PGx panel testing included participant demographics, potential genotype actionability, current actionability of gene-drug pairs, prevalence of drug-drug-gene interactions that affect CYP2D6 clinical phenotype (phenoconversion), and post-test participant perspectives. When assessing potential actionability, genotypes were considered actionable if a potential drug or dose change was suggested by

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TABLE 3 Pharmacogenetic results.

Phenotype/Genotype	*n* (%) total *n* = 61
CYP2C9	
IM	25 (41%)
NM	36 (59%)
CYP2C19	
PM	2 (3%)
IM	12 (20%)
NM	30 (49%)
RM	16 (26%)
UM	1 (1.5%)
CYP2C Cluster	
G/G	46 (77%)
G/A	13 (21%)
A/A	2 (2%)
CYP2D6	
PM	1 (1.5%)
IM	7 (11%)
NM	52 (85%)
Unable to genotype	1 (1.5%)
CYP3A5	
NM	11 (18%)
PM	50 (82%)
CYP4F2	
*1/*1	27 (44%)
*1/*3	27 (44%)
*3/*3	7 (11%)
SLCO1B1	
Decreased function	15 (24.5%)
Normal function	46 (75.5%)
VKORC1	
G/G	22 (36%)
G/A	27 (44%)
A/A	12 (20%)

PM, poor metabolizer; IM, intermediate metabolizer; NM, normal metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer

CPIC guideline recommendations for any medication, regardless of what medications the participant was currently taking. Current actionability of gene-drug pairs were determined based on the participant's clinical phenotype and currently active panel drug; if the pair's associated guideline recommendations published by CPIC suggested a drug or dose change, it was considered actionable.

Results

A total of 244 eligible UF Health patients were identified through initial EHR review. Contact was successfully established with 66% (*n* = 162) of these patients (Figure 2). Upon establishing contact and confirming eligibility, 17 patients were excluded from the pilot as they were no longer on a panel drug (*n* = 10), no longer enrolled in a

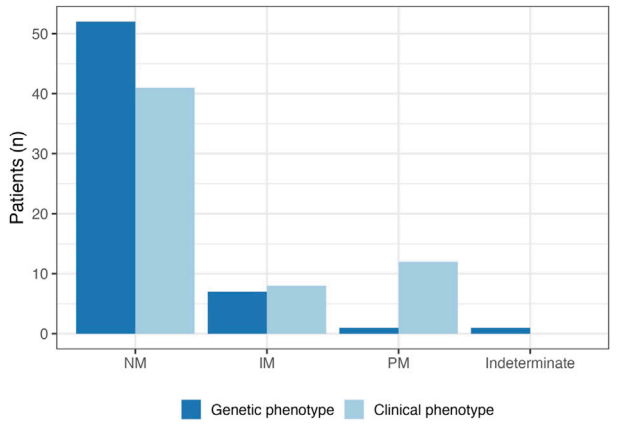

FIGURE 3
CYP2D6 genotype-based and clinical phenotype. A total of 14 patients were taking strong or moderate CYP2D6 inhibitors. Almost all (n = 13) experienced phenoconversion to intermediate metabolizer (IM) or poor metabolizer (PM) as a result; clinical phenotypes are represented by the light blue bars. The prevalence of CYP2D6 PMs increased from 1.5% to 20% after accounting for phenoconversion.

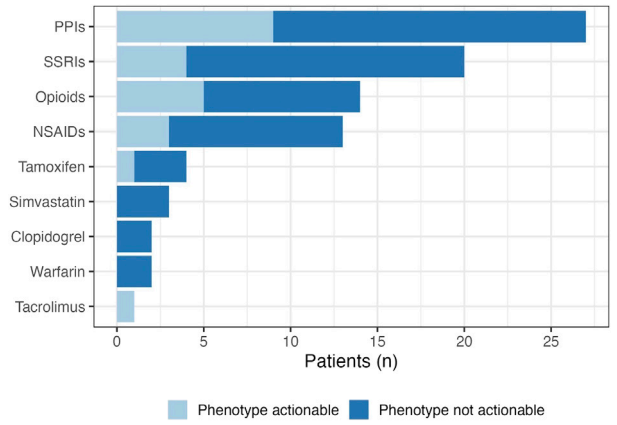

FIGURE 4
Pharmacogenetic drug class at baseline. The prevalence of actionable gene-drug pairs are represented by the light blue section of the bars. Actionability was determined based on the patient's clinical phenotype, and the associated CPIC guideline recommendations for relevant panel drugs.

GatorCare benefit plan (n = 6), or already had PGx testing (n = 1). Of the 145 confirmed eligible patients, 76% (n = 110) agreed to participate. The majority of patients (55%; n = 60) agreed to participate through a phone call; 50 agreed to participate after the initial phone call, and an additional 10 patients agreed after a follow-up call. Most other patients (43%; n = 47) agreed to participate in the pilot by responding to the initial MyChart message. One provider requested DNA sample collection materials at their clinic as they opted to self-identify eligible patients. The remaining 3% (n = 3) agreed to participation while at an in-person UF Health clinic visit with this provider after eligibility was confirmed. No patients opted to participate as a direct result of the physically mailed letter. The remaining 24% (n = 35) of the confirmed eligible patients with whom we established contact declined participation. Many of those who declined expressed a fatigue with the healthcare system explaining that they simply did not have time for more testing, while others simply did not want to participate in PGx testing, especially if they did not understand it.

Of the 110 patients who opted to participate, 55% (n = 61) provided their PGx sample and received PGx results. The majority of these patients (n = 58) returned their mailed DNA collection kit, while those enrolled in person completed testing in person the same day. The average turnaround time from outbound mailing of DNA collection kits to the date the results were reported was just over 1 month (30.9 ± 14.6 days), and some (n = 3) outliers took up to 3–4 months to return their DNA collection kits. Once returned and delivered to the UF Health Pathology lab, the average turnaround time for sequencing is 3–7 days. Patients who provided their DNA sample were mostly female (64%), White (87%), and were an average of 51 years old (Table 2). All 61 patients received PGx results for the 8 genes included on the panel except for one patient whose CYP2D6 genotype returned as "indeterminate." Nearly 89% of patients had a minimum of one potentially actionable phenotype as determined by their genotype-based phenotype.

A complete breakdown of participant genotype/phenotype results can be found in Table 3. A total of 23% of patients (n = 14) were taking a strong or moderate CYP2D6 inhibitor, and all but one participant experienced phenoconversion of their genotype-based phenotype to a clinical phenotype of either CYP2D6 poor or intermediate metabolizers as a result (Figure 3).

Of the 61 patients who participated, there was a combined total of 86 active panel drug prescriptions at time of enrollment. Most patients were on one panel drug, however nearly 40% of patients had at least two active panel drugs in their medication list at baseline. It is worth noting that three patients thought to be eligible were realized to be on zero active panel drugs following the return of their DNA collection kit. This was largely due to patients being on topical tacrolimus rather than the oral formulation which was reported by these patients during telephone follow-up conversations. The most common drug class of active panel drugs were proton pump inhibitors (PPIs; n = 27 patients with an active PPI prescription), followed by selective serotonin reuptake inhibitors (n = 20). Pain management was also relevant with 14 active panel drugs being opioids, and 13 being nonsteroidal anti-inflammatory drugs (Figure 4). The most common active panel drug was omeprazole (n = 14), followed by tramadol (n = 9), escitalopram (n = 8) and pantoprazole (n = 8). Just over a quarter (n = 23) of gene-drug pairs were currently actionable, seven of which have at least a moderate level of evidence supporting them. The most common actionable gene-drug pair (n = 5) included omeprazole in CYP2C19 poor or intermediate metabolizers. This is followed closely (n = 4) by pantoprazole in CYP2C19 intermediate or ultrarapid metabolizers. An additional 16% of active panel drugs (n = 14) were PPIs in patients who are CYP2C19 normal or rapid metabolizers. This gene-drug pair is worth noting as it may become actionable in the future based on indication (Lima et al., 2021).

Post-test follow-up phone calls were successful with 39% (n = 24) of patients with PGx results. As displayed in Figure 5, follow-up calls revealed that more than 80% of patients would describe the

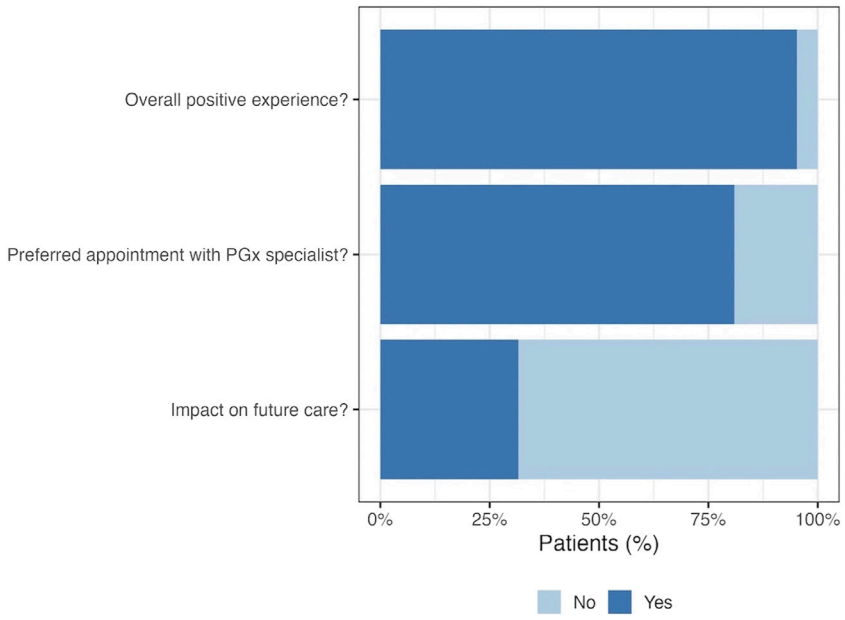

FIGURE 5
Patient perspective at post-test call. Post-test follow up phone calls were made to patients with pharmacogenetic results in order to gain insight into participant perceptions on the pilot program. Twenty-four patients completed the post-test call, the majority of which expressed preferring to have had an appointment with a PGx specialist following testing.

process as an overall positive experience, but 71% said they would have preferred an appointment with a PGx specialist to help explain and interpret their genetic results. Only 25% of contacted patients expressed feeling like their PGx test results may have a potential impact on their future care, and many of those who did not perceive a potential impact noted they and/or their physician did not understand their results.

Discussion

This implementation pilot provided new insights into patient attitudes about PGx testing and the specific challenges of implementing a PGx testing program through an employer-sponsored medical plan. Among the eligible UF Health patients screened for inclusion in the pilot, 76% were offered PGx testing with no additional direct costs. Those who declined to participate cited reasons such as lack of time or interest, uncertainty about the benefits of PGx testing, and concerns about privacy and the potential for misuse of their genetic data. This suggests that, from a patient's perspective, cost is not the only relevant barrier to implementing PGx testing. Panel-based PGx testing was ordered using a partially preemptive approach, in which we targeted patients with current prescription(s) for medications with relevant gene-drug associations. While this approach shares elements with reactive testing, it differs in that patients tested reactively are often at risk for or have experienced an adverse event or treatment failure, while patients tested using a partial preemptive approach may not have been having any issues with their medications. This may have diminished the urgency of completing the test. These results suggest that preemptive testing could potentially serve as a barrier to PGx testing based on lack of understanding of the test's purpose by patients and providers. Although we aimed to take cost, uncertainty about insurance coverage, and in-person clinic visits out of the equation, less obvious implementation barriers were revealed in this implementation. These challenges, however, help identify important pitfalls that can be useful for the success of future preemptive PGx implementation programs. A description of implementation challenges encountered and lessons learned are described in Table 4.

While the patients may not have had any known medication-related problems at the time of test initiation, the purpose of this pilot was to complete testing so that the results would be available should the patient need them in the future. It is highly likely that the results will be impactful in the future as 89% of patients had one potentially actionable genotype-based phenotype. While the majority of tested patients have a clinically useful PGx test result, only 25% of patients contacted for follow-up expressed feeling that their results would have an impact on their future care. This suggests that patients will miss out on the benefits of PGx testing if they do not understand what their results mean. Patients noted that they did see their results in their patient portal, but many expressed not understanding the results. At the time of this implementation patient-friendly language around the results in the patient portal did not exist; we have since updated the portal to provide basic information. Even with this update, it is most beneficial for a patient to be educated on their results by a PGx-specialist, or their prescriber, if knowledgeable about PGx results. Some patients revealed that they did discuss the PGx results with their primary care physician, but their physician did not understand the results enough to apply them to their care. Although a consult note was placed in patients' EHRs based on their current medication lists and

TABLE 4 Summary of relevant implementation challenges encountered and potential solutions.

Category	Challenge	Potential solution or lesson
Patient participation	Limited understanding of the purpose of testing • Low return rate of DNA collection kits	• Enhanced pre-test education for patients is needed. This would provide more background information explaining who can benefit and which medications are informed by testing • Tests were provided free of charge. Patients may respond better with a financial or other type of buy-in to the testing process • Post-test patient-friendly educational handouts describing results help bridge this knowledge gap with patients
	Low participation rate • Response to participation requests was relatively low • Post-test calls were only successfully completed in a handful of patients	• A verbal conversation can help mitigate patients' concerns with testing. The majority of patients with established contact did ultimately agree to participate • Pre-emptive testing may inherently serve as a barrier to implementation. Without any current drug therapy problems, the purpose of testing may be unclear • Collecting information on disease state and current response to medications in future implementation projects would provide insight into which patients are more likely to participate in PGx testing
	Lack of perceived benefit • Low perceived impact on care	• Improving post-test education by offering consultation with a pharmacist, for example, could improve perceived benefit of testing • While most participants did not think PGx testing would have an impact on their care, it is encouraging to see the majority had a desire to understand their results better
Provider education	Knowledge gaps and varying levels of comfort with interpreting results • Some patients reported their provider did not know how to use their PGx results	• Utilizing pharmacist consult notes to help providers use PGx information with more confidence is needed • Having a mechanism for requesting a consult or pharmacogenetic test interpretation integrated into the electronic health system is also beneficial
EHR	EHR-reported medication lists are often inaccurate or incomplete	• Patient engagement is needed to obtain an accurate medication history in most EHR systems. This can improve the ability to provide relevant medication recommendations
	The system may not be set up to access pharmacogenetic results easily	• Pharmacogenetic results were previously only reported as a lab value, which is not optimal if a non-expert is trying to interpret results. Our institution has since implemented a separate tab within the EHR entitled "Genomic Indicators". Providers can now use this tab to view patient-specific recommendations for medications informed by PGx testing

EHR, electronic health record; *BPA*, best practice advisory.

genetic results, most patients still expressed that they would have preferred an opportunity to speak with a PGx-trained pharmacist to help understand results. Additionally, more than 20% of patients with PGx test results were expected to experience drug-induced CYP2D6 phenoconversion based on their medication lists. The prevalence of phenoconversion further emphasizes the need for PGx-trained pharmacists to help with the accurate interpretation of genetic results that takes concomitant therapy into account.

Another challenge encountered was the reliance on incomplete or inaccurate medication lists in patients' EHRs. Despite confirming eligibility at time of initial contact, the process failed to identify all ineligible patients. Three patients thought to be eligible were enrolled and received pharmacogenetic testing, but it was later realized that these patients did not take any panel drugs. This suggests that automating phenoconversion would not be feasible and further supports pharmacist interpretations. The use of EHR medication lists also has the potential to miss any over-the-counter medications affected by pharmacogenetics, such as certain NSAIDs or PPIs. These issues highlight the importance of engaging with patients to provide them with accurate and relevant interpretation of PGx test results. Engaging with patients earlier could potentially confirm medication lists, but this is not always possible in an automated workflow.

There was a clear desire by patients to better understand their genetic results and relevant medications is not novel and is indicative of a need for the expansion of pharmacist-led PGx clinics like those already being implemented at several institutions across the United States (Duarte et al., 2021). In fact, since the completion of this pilot program the UF College of Pharmacy and Center for Pharmacogenomics and Precision Medicine have partnered with UF Health to launch MyRx, 2022 (https://myrx.ufhealth.org/), a clinical PGx consultation service utilizing online visits with PGx-trained pharmacists to educate patients on their test results.

Implementations such as this pilot program serve as an important tool for identifying ways to bridge the information gap between patients, providers, and PGx-specialists. This engagement may continue to become easier as the evidence base supporting preemptive or partially preemptive PGx testing's impact on clinical outcomes continues to grow. The recently published PREPARE study conducted a large, open-label, prospective, cluster-randomized-controlled implementation study of a 12-gene PGx panel (Swen et al., 2023). The investigators observed a 30% reduction in patient-reported clinically relevant adverse drug reactions with the use of PGx-guided prescribing. The PREPARE study included close to 7,000 patients; however, study results are

limited by a lack of diversity. There is currently still a need for more diverse PGx implementation studies to properly identify and address implementation barriers unique to these populations.

Limitations

Lack of diversity is a common limitation in pharmacogenetic trials, and is relevant to our pilot program, as well. With an already relatively small sample size and 87% of enrolled patients being White, it is likely that the experiences of underrepresented populations in the United States are not well represented by our patient population. The small sample size of this pilot program could have limited our ability to fully assess the challenges identified above. A larger patient population in future implementation programs would likely provide further insight into relevant challenges. Additionally, because this pilot was conducted during the COVID-19 pandemic, patient attitudes and behaviors may have limited generalizability due to potential hardships associated with the pandemic.

There are several limitations to the GatorPGx Panel used for pharmacogenetic testing in this pilot. Eight genes were included in the panel that were thought to have a potential impact on a large majority of patients. However, there are genes with a high level of evidence and prescribing guidance available that were not tested for (e.g., DPYD, UGT1A1). It is also possible that some patients may have had a single nucleotide polymorphism (SNP) present that went undetected by the GatorPGx Panel. Only SNP's on a predetermined list associated with the assay (Supplementary Figure S3) were able to be detected. If one of the listed SNPs was not identified, then the resulting genotype would default to the wildtype "*1" allele. This has potential to misclassify patients with altered enzyme function as a normal metabolizer.

The curated list of panel drugs resulted in relevant limitations to this pilot program, as well. Not all relevant medications (e.g., atorvastatin) that can be informed by pharmacogenetic testing were included, and therefore some patients who were on a medication relevant to PGx may have been missed. Additionally, certain panel drugs (e.g., rabeprazole) are no longer considered to be informed by pharmacogenetic testing based on updated evidence and CPIC guidelines published after the start of this pilot program (Lima et al., 2021; Cicali et al., 2023).

There are several limitations regarding the enrollment, contact, and follow-up in this pilot. Eligibility was determined based on active medications in the patient's EHR, which may have been inaccurate or incomplete. Inaccurate medication lists may have resulted in the exclusion of patients who were actually eligible, or *vice versa*. All follow-up phone calls were conducted and evaluated by a single author, which may limit the validity of their interpretation. Although no formal parameters were set up to guide the evaluation of patient responses, the same three questions were asked to everyone and an effort was made to keep consistency. Regardless of these described limitations, we were still able to learn key lessons from this pilot implementation to guide future implementations.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

EC, KW, LC, KN, BA, and JS contributed to conception and design of the pilot. RD and MN organized the data and performed the statistical analysis. MN wrote the first draft of the manuscript. RD, EC, KW, and BA, wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

JS was employed by University of Florida Health.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1249003/full#supplementary-material

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Pharmacogenetics and phenoconversion: the influence on side effects experienced by psychiatric patients

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Introduction: Preventing side effects is important to ensure optimal psychopharmacotherapy and therapeutic adherence among psychiatric patients. Obtaining the pharmacogenetic profile of *CYP2C19* and *CYP2D6* can play an important role in this. When the genotype-predicted phenotype shifts because of the use of co-medication, this is called phenoconversion. The aim was to study the influence of the pharmacogenetic (PGx) profile and phenoconversion on side effects experienced by psychiatric patients.

Methods: A retrospective cohort study was performed using data from 117 patients from a psychiatric outpatient clinic. Patients were genotyped with a psychiatric PGx panel and side effects were evaluated using the *Udvalg for Kliniske Undersølgelser* side effects rating scale (UKU).

Results: Of all patients, 10.3% and 9.4% underwent phenoconversion (any shift in predicted phenotype) for *CYP2C19* and *CYP2D6* respectively. No significant associations were found between the phenotype and UKU-score. 75% of the patients with an Intermediate metabolizer (IM) or Poor metabolizer (PM) phenoconverted phenotype of *CYP2C19* experienced nausea and vomiting compared to 9.1% of the Normal metabolizer (NM) and Ultrarapid metabolizer (UM) patients ($p = 0.033$). 64% of the patients with an IM or PM phenoconverted phenotype of *CYP2D6* experienced the side effect depression compared to 30.4% NMs and UMs ($p = 0.020$). *CYP2D6* IM and PM patients had a higher concentration-dose ratio than NM patients ($p < 0.05$).

Discussion: This study underlines the importance to consider phenoconversion when looking at a patient's genotype. This is important for a better prediction of

Abbreviations: CAD, Coronary artery disease; DGI, Drug-gene interaction; DPWG, Dutch Pharmacogenetics Working Group; EPS, Extrapyramidal symptoms; LL-clinic, "Body and Life" outpatient clinic (Dutch: *Lijf en Leven*); LL-patients, Patients from "Body and Life" outpatient clinic; P-CYP2C19, Phenotype CYP2C19 after phenoconversion; P-CYP2D6, Phenotype CYP2D6 after phenoconversion; UKU, *Udvalg for Kliniske Undersølger* side effects rating scale.

the phenotype and preventing possible side effects under a specific psychopharmacotherapy.

KEYWORDS

pharmacogenetics, phenoconversion, side effects, psychiatric drugs, CYP2C19, CYP2D6

1 Introduction

A high prevalence of polypharmacy is seen among psychiatric patients (Hefner et al., 2020). Polypharmacy, the use of five or more drugs, is often associated with drug-drug interactions and the risk of side effects. Preventing these side effects is important to ensure optimal psychopharmacotherapy and therapeutic adherence (Bousman et al., 2021).

The use of pharmacogenetics (PGx) contributes to individual patient treatment and can play an important role in preventing side effects (Sharp et al., 2019). PGx can distinct the different genetic variants of genes encoding for cytochrome P450 enzymes (CYP) such as CYP2C19 and CYP2D6 with a different metabolic capacity (KNMP, 2022a; KNMP, 2022b). A patient's genotype can be translated into the following predicted phenotypes: normal metabolizer (NM), intermediate metabolizer (IM), poor metabolizer (PM) or ultrarapid metabolizer (UM) (Brouwer et al., 2022). Different consortia such as the Dutch Pharmacogenetics Working Group (DPWG) or the Clinical Pharmacogenetics Implementation Consortium (CPIC) have written guidelines regarding dose and pharmacotherapeutic recommendation for each genotype with an actionable drug-gene interaction (DGI) (Abdullah-Koolmees et al., 2021).

CYP2C19 and CYP2D6 are responsible for the metabolism of many psychiatric drugs (Abdullah-Koolmees et al., 2021; Brouwer et al., 2022). In addition, these are also highly polymorphic enzymes and therefore pharmacogenetic advise on these DGIs are widely available (Bousman and Dunlop, 2018; Hahn and Roll, 2021). Furthermore, PGx can help improve and optimize pharmacotherapy for individual patients using psychiatric drugs. Other CYP-enzymes such as CYP1A2, CYP2C9 and CYP3A4 can also play a role in the metabolism of psychiatric drugs. However, the impact of their genotypes on the pharmacokinetics of commonly used psychiatric drugs is less distinct as compared to the effect of *CYP2C19* and *CYP2D6* genotypes. Previous studies have reported that patients with a predicted phenotype of PM for either *CYP2C19* or *CYP2D6* have a higher risk of side effects (Chou et al., 2000; Kobylecki et al., 2009; Mrazek et al., 2011). However, in other studies no association between the genotype and the development of side effects has been observed (Peters et al., 2008; Hodgson et al., 2015).

Other non-genetic factors, such as co-medication, also influence the patient's phenotype, i.e., a patient's metabolic capacity (Klomp et al., 2020). This can have a significant impact, especially on patients with polypharmacy. This phenomenon, where the predicted metabolic capacity shifts because of the use of co-medication or other non-genetic factors, is called phenoconversion (Hahn and Roll, 2021). In this study, phenoconversion by co-medication will be taken into account.

Research shows that CYP-inhibition or CYP-induction by co-medication often has the greatest influence on NMs and IMs, causing a change in the drug exposure (AUC) (Bahar et al., 2017). For instance, it has been shown that patients using (es)citalopram are more prone to a dose reduction or switching to another antidepressant when there is a drug-drug-interaction combined with a DGI with *CYP2C19* (MuhA et al., 2020). However, the relationship between side effects, pharmacogenetic profile and phenoconversion remains to be studied. The aim was to study side effects experienced by psychiatric patients and to identify risk factors including but, not limited to, pharmacogenetic profile of *CYP2C19* and *CYP2D6* and phenoconversion.

2 Materials and methods

2.1 Study design and population

A retrospective cohort study was performed using the data of the "Body and Life" project (Dutch: *Lijf en Leven*), for which a non-WMO acknowledgment from the Medical Research Ethics Committee Utrecht has been authorized (number 19-447/C). All patients gave written informed consent. For this study, psychiatric patients were enrolled from the outpatient clinic also named "Body and Life" (Dutch: *Lijf en Leven*, LL-clinic) of the department of psychiatry from the University Medical Center Utrecht (UMC Utrecht) in the Netherlands (UMC Utrecht, 2022a). Patients were excluded if no genotyping was performed. Patients enrolled between February 2018 and March 2022 were included in the analysis. Data was extracted from the electronic health record.

For the comparison of the distribution of the genotypes, control populations were used. For the control population of *CYP2C19*, a group of 820 coronary artery disease (CAD) patients who underwent elective coronary stenting was used. For the control population of *CYP2D6*, a group of 134 healthy controls recruited from hospital personnel was used. These patients have been genotyped as part of other studies, which have been approved by Medical Research Ethics Committee of St. Antonius Hospital Nieuwegein, the Netherlands.

2.2 Drug classification and phenoconversion

The current drug use was documented. Within the drugs used, CYP-substrates as well as CYP-modulators for CYP2C19 and CYP2D6 were identified. CYP-substrate users were defined as a patient who uses a CYP2C19- or CYP2D6 substrate which has a psychiatric indication and where a therapeutic recommendation is given for the DGI by the DPWG (Abdullah-Koolmees et al., 2021; KNMP, 2022c). If no advice was given for a substrate, a literature search was conducted to see if the DGI was of clinical relevance. This was only the case for diazepam, which is considered a CYP2C19-substrate with a pharmacogenetic interaction. (Qin et al., 1999; Skryabin et al., 2020; KNMP, 2022d).

TABLE 1 Phenoconverted phenotype for CYP2D6 and CYP2C19 based on concomitant use of inhibitor/inducer. The presented phenotypes are what the genotype-predicted phenotypes will convert to when a moderate inhibitor, strong inhibitor or inducers is taken concomitantly.

Genotype-predicted phenotype	Moderate inhibitor	Strong inhibitor	Inducer (only for CYP2C19)
PM	PM	PM	PM
IM	PM	PM	NM
NM	IM	PM	UM
UM	NM	IM	UM

Abbreviations PM, poor metabolizer; IM, intermediate metabolizer; NM, normal metabolizer; UM, ultrarapid metabolizer.

A CYP-modulator was defined as a drug that has a moderate or strong inhibitory or inducing effect on CYP2C19 and/or CYP2D6 (Cicali et al., 2021). There are no known inducers for CYP2D6 (Just et al., 2021). Relevant inhibitors and inducers can be found in Supplementary Material S1. In this study, phenoconversion was defined as the shift of a patient's phenotype based on the use of co-medication consisting of CYP-modulators. The genotype-predicted phenotype was adjusted to a phenoconverted phenotype (*P-CYP2C19* and *P-CYP2D6*) according to Table 1 (Hahn and Roll, 2021; Just et al., 2021). Phenotypes were classified in the next lower activity phenotype using a moderate inhibitor and an even lower activity phenotype when using a strong inhibitor. For example, a patient who is a *CYP2D6* NM but uses fluoxetine, a strong CYP2D6-inhibitor, is a *P-CYP2D6* PM. If a patient used a CYP2C19-inducer, a patient was classified into the next higher activity phenotype. Only PMs kept poor activity because increased synthesis of "loss of function proteins" does not change the drug clearance and thus the phenotype.

2.3 Genotyping

DNA-diagnostics were performed at *Erasmus MC clinical laboratory* in Rotterdam, Netherlands (Erasmus, 2022). Genotypes were translated to corresponding phenotypes recognized by the DPWG (Abdullah-Koolmees et al., 2021; KNMP, 2022a). Patients were tested for *CYP1A2, CYP2C9, CYP2C19, CYP2D6* and *CYP3A4*, but only the genotypes of *CYP2C19* and *CYP2D6* were used. *CYP1A2* was not included because there were no actionable DGIs according to the DPWG. *CYP2C9* was not included because no patient used a CYP2C9-metabolized psychiatric drug with a relevant DGI and for *CYP3A4* were no PMs identified (only phenotype with actionable PGx recommendation).

2.4 Side effect registration

Side effects were evaluated using the *Udvalg for Kliniske Undersølgelser side effects rating scale (UKU)* for the registration of side effects of psychotropic drugs (Lingjærde et al., 1987). In the UKU, every side effect is rated in a four-point scale, and all scores were added together to get a total score. A higher score implies a higher rate of side effects or more severe side effects. An adapted version of the UKU-rating scale specifically for the LL-clinic was used, from which the extrapyramidal symptoms were excluded from the category neurologic side effects and can be found in the Supplementary Material S2. The UKU-questionnaire was conducted orally by a nurse specialist via a semi structured interview with the patient.

2.5 Concentration-dose ratios

Next to a blood sample for the DNA-diagnostics, there was also a sample send to the *pharmacy laboratory of UMC Utrecht* for determination of drug levels in plasma (UMC Utrecht, 2022b). This was done for all psychiatric drugs a patient used at the time of measurement. For each patient a blood sample was taken in the morning, with medication taken the night before but not in the morning. Based on the drug concentration (in μg/L) and the registered dose (in mg), the concentration-dose ratio (CD-ratio) was calculated in μg/l/mg for further analysis. Only the drugs with at least two users in the different phenotypic groups were included for further analysis.

2.6 Statistical analysis

Continuous variables were presented as mean and standard deviation (median and range if non-normal distributed) and categorical variables as frequency and percentage. Comparisons of normal distributed continuous variables were performed with a student's T-test. For non-normal distributed variables, a Mann-Whitney U-test was used or a Kruskal Wallis test if there were more than two groups. For categorical variables, a chi-squared test was used. Only CYP-substrate users were included in the phenotype-specific analysis and NM was seen as the reference phenotype.

Associations between the UKU-score and patient characteristics were analyzed using binary logistic regression. Phenotypes were combined, i.e., NM with UM and IM with PM, because of the small sample size per phenotype group. Although there are known distinct PK differences between these phenotypes, it is hypothesized that the IMs and PMs will experience more side effects than the NMs and UMs when receiving standard doses. For the analysis of the UKU-score the score was divided into three categories (low, moderate and high) based on tertiles. An univariate as well as a multivariate analysis was performed, adjusting for body mass index (BMI), psychiatric diagnosis and polypharmacy.

The comparison of the prevalence of specific side effects was only done for CYP-related side effects, which were determined for a project of the *Ubiquitous Pharmacogenomics Consortium*.

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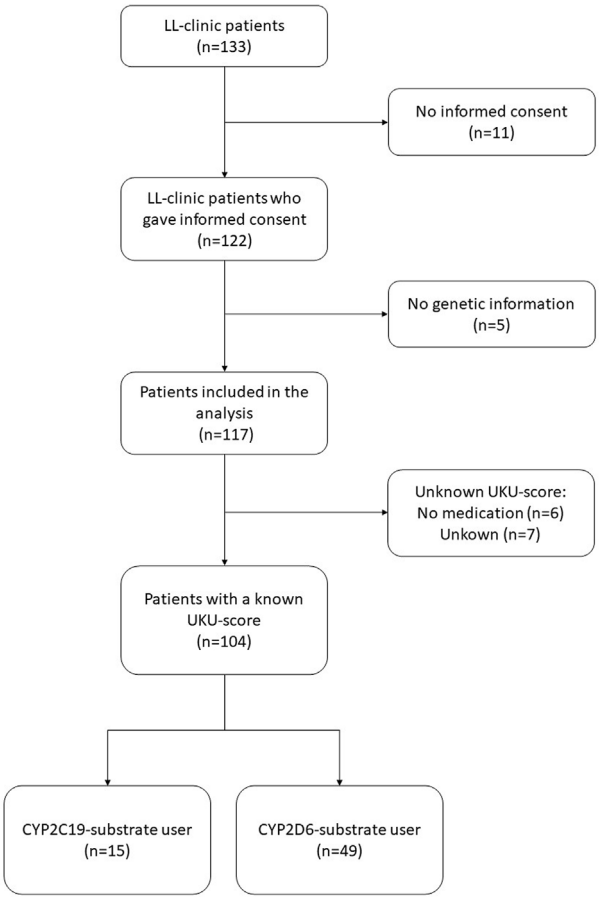

FIGURE 1
Selection of LL-clinic patients included into this study. Abbreviations *LL-patients* patients from "Body and Life" outpatient clinic; *UKU* Udvalg for Kliniske Undersølger side effects rating scale.

Five researchers assessed each side effect based on clinical studies, the Summary of Product Characteristics and expert-opinions. The outcomes of the assessments [unpublished], i.e., whether specific side effects of drugs are genotype dependent, was used. Of the UKU-questionnaire, 33 of the 39 side effects were considered CYP-related (see Table 5; Table 6 for the included side effects).

A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics (Version 26.0.0.1, IBM Corp., Armonk, NY, United States).

3 Results

In total, 117 LL-patients were eligible for this study, of which 104 (88.9%) LL-patients filled in the UKU-questionnaire and could be included in the analysis (Figure 1). Of the 104 LL-patients who were included in the analysis, 15 (14,4%) LL-patients concomitantly used a CYP2C19-substrate and 49 (47,1%) a CYP2D6-substrate. Table 2 shows the baseline characteristics of the study population. Figure 2 compares the distribution of the different genotype-predicted phenotypes of *CYP2C19* and *CYP2D6*. The proportion of UMs in *CYP2C19* was significantly larger for the LL-patients (8.5%) compared to the CAD-patients (4.0%).

Approximately 10% of the LL-patients underwent phenoconversion for either *CYP2C19* or *CYP2D6* (Table 2). The comparison of the distributions of CYP2D6 before and after phenoconversion, revealed a significant difference between the proportions of PMs (Figure 2). In the genotype-predicted phenotypes, 6.8% of the patients were a PM of *CYP2D6*, which was 16.2% of the *P-CYP2D6* patients (p < 0.05). No significant differences were found within *CYP2C19* phenotype groups.

Comparing the total UKU-score, significant differences were found for the main diagnosis when comparing a psychotic diagnosis category (median score 13) to non-psychotic category (median score 22, p < 0.05) (Table 3). There was also a significant difference in the total UKU-score considering polypharmacy (patients without polypharmacy had a median score of 11, patients with polypharmacy a median score of 20, p < 0.05). The UKU-score was higher for patients with BMI over 40, but this was not a significant difference (p = 0.064; median BMI < 30 is 13, median BMI 30–40 is 15 and median BMI > 40 is 23.5). No significant differences were found for age and gender.

Patients with a non-psychotic main diagnosis had an odds ratio (OR) of 2.60 (95% CI: 1.04–6.54; adjusted OR 2.43; 95% CI: 0.90–6.55, Table 4) for higher UKU-score. Patients with polypharmacy had an OR of 4.47 (95% CI: 1.90–10.53; adjusted OR 4.26; 95% CI: 1.76–10.32) for higher UKU-score. No other statistical differences between UKU-score and covariates were found.

There was no increase of the total UKU-score associated with the genotype-predicted phenotypes of *CYP2C19* and *CYP2D6* (Table 3; Table 4). For *P-CYP2D6*, there was a significant difference between the total UKU-score of the NM (median 14) and UM (median 43.5, p < 0.05). There were no significant differences for IM (median 19.5) and PM (median 15). For *P-CYP2C19*, no increase in total UKU-score was seen considering the different phenotypes.

No significant differences were seen in the prevalence of certain side effects comparing the different genotype-predicted phenotypes of *CYP2C19* (Table 5) and *CYP2D6* (Table 6). Considering phenoconversion, several differences were noted. For *P-CYP2C19*, there was a higher prevalence of nausea and/or vomiting in the IM and PM group (75.0%) compared to the NM and UM group (9.1%, p < 0.05, Table 5). There were no further side effects with significant between-group differences. Comparing the prevalence of specific side effects and *P-CYP2D6*, there was a significant difference in the prevalence of depression (Table 6). 30.4% of the NMs and UMs experienced this side effect compared to 64.0% of the IMs and PMs. The IMs and PMs also experienced a higher rate of increased dream activity (48.0%) compared to the NMs and UMs (21.7%), but this result was not statistically significant (p = 0.057). The same is true for sleepiness, which 76.0% of the IMs and PMs experienced compared to 52.2% of the NMs and UMs (p = 0.085), and nausea and/or vomiting, which 36,0% of the IMs and PMs experienced compared to 13,0% for the NMs and UMs (p = 0.067). More patients in the NM and UM group of *P-CYP2D6* (13.6%) experienced gynecomastia, compared to 0% of the IMs and PMs. However, this was also not statistically significant (p = 0.095). Other side effects did also not show any significant differences.

TABLE 2 Baseline characteristics of LL-patients.

	Patients
Total	117
Gender (%)	
Male	46 (39.3)
Female	71 (60.7)
Age—in years (mean ± SD)	42.5 ± 11.8
Weight—in kg (mean ± SD)	109.3 ± 28.7
Height—in cm (mean ± SD)	174.2 ± 10.5
BMI—in kg/m^2 (mean ± SD)	36.0 ± 9.4
BMI classification (%)	
Underweight (< 18,5)	1 (0.9)
Normal weight (18,5–24,9)	11 (9.4)
Overweight (25–29,9)	18 (15.4)
Obese (30–39,9)	55 (47)
Morbid obese (BMI ≥ 40)	31 (26.5)
Main diagnosis (%)	
Schizophrenia	43 (36.8)
Personality disorder	11 (9.4)
Bipolar mood disorder	29 (24.8)
Depressive mood disorder	13 (11.1)
Anxiety disorder	2 (1.7)
PTSD	6 (5.1)
Neurodevelopmental disorder	8 (6.8)
Somatic symptom disorder	1 (0.9)
Addiction	1 (0.9)
Eating disorder	2 (1.7)
Other	1 (0.9)
Main diagnosis grouped (%)	
Psychotic (schizophrenia and bipolar disorder)	72 (61.5)
Non-psychotic (depression and others)	45 (38.5)
Amount of drugs in use (median [range])	5 [0–16]
Polypharmacy (≥5 drugs in use) (%)	
Yes	68 (58.1)
No	49 (41.9)
CYP2C19-substrate in use (%)	
Yes	17 (14.5)
No	100 (85.5)
CYP2D6-substrate in use (%)	
Yes	52 (44.4)
No	65 (55.6)

(Continued on following page)

TABLE 2 (*Continued*) Baseline characteristics of LL-patients.

	Patients
CYP2C19-modulator in use (%)	
No modulator	85 (72.6)
Weak inhibitor	20 (17.1)
Moderate inhibitor	6 (5.1)
Strong inhibitor	3 (2.6)
Inducer	3 (2.6)
Phenoconversion CYP2C19 (%)	
Yes	12 (10.3)
No	105 (89.7)
CYP2D6-inhibitor in use (%)	
No inhibitor	91 (77.8)
Weak inhibitor	15 (12.8)
Moderate inhibitor	–
Strong inhibitor	11 (9.4)
Phenoconversion CYP2D6 (%)	
Yes	11 (9.4)
No	106 (90.6)

Abbreviations *BMI*, body mass index; *SD*, standard deviation; *PM*, poor metabolizer; *IM*, intermediate metabolizer; *NM*, normal metabolizer; *UM*, ultrarapid metabolizer.

For the comparison of the CD-ratio, the number of patients was sufficient for the analysis of CYP2D6-substrates aripiprazole, risperidone, haloperidol and venlafaxine. From the data in Figure 3, it was apparent that the concentration-dose ratio of aripiprazole is significantly higher for IMs and PMs than NMs with (7.55 ± 3.46 μg/l/mg versus 16.03 ± 4.92 μg/l/mg) and without (8.98 ± 5.16 μg/l/mg versus 15.43 ± 5.25 μg/l/mg) considering phenoconversion. For risperidone, haloperidol and venlafaxine no statistic significant differences were seen.

4 Discussion

This study shows that in 10% of the psychiatric patients any form of phenoconversion, where the predicted phenotype shifts based on genotype and co-medication, occurred. It also plays a role in the side effects experienced by these patients. This study shows that specific side effects such as nausea and depression are more prevalent in patients with an IM or PM phenoconverted phenotype of *CYP2C19* and *CYP2D6*.

In this study population, there were significantly more *CYP2C19* UM patients compared to the control population consisting of CAD-patients. In the Dutch Caribbean population, in which the phenotype distribution is comparable to Caucasians, a study found no differences at all in the prevalence of specific *CYP2D6* or *CYP2C19* phenotypes in psychiatric patients (Koopmans et al., 2017). This difference may be explained by the fact that the UMC Utrecht is a tertiary care center, to which patients are only referred if the treatment in the first or secondary line of care was not adequate. In a previous study in an American tertiary psychiatric hospital, a higher prevalence of genetic variants leading to a phenotype other than NM was seen (Ruaño et al., 2008). Looking at the distribution of phenotypes before and after phenoconversion, there were specifically more *P-CYP2D6* PM patients. These findings seem to be consistent with the existing literature (Preskorn et al., 2013; Mostafa et al., 2019). This shows it is important to consider phenoconversion when predicting a patient's phenotype. For *CYP2C19* and *CYP2D6*, the phenotype influences the efficacy and tolerability of antidepressants and consequently there are different pharmacotherapeutic recommendations for each specific genotype-predicted phenotype (Gressier et al., 2015; Brouwer et al., 2022; Campos et al., 2022). There are also pharmacotherapeutic recommendations available for antipsychotics (Beunk et al., 2023). So, if the genotype-predicted phenotype shifts because of phenoconversion, it is possible that other recommendations are given.

Based on the total UKU-score, no statistically significant associations were found between the genotype-predicted or phenoconverted phenotype and amount and/or severity of side effects. However, the OR of the *CYP2D6* phenoconverted phenotype was 1.31 and higher than 0.74, the OR of the *CYP2D6* genotype-predicted phenotype. No comparison could be made for *CYP2C19* because no logistic regression could be performed in the genotype-predicted group. In other studies, it has also been seen that there is a stronger association between phenoconverted phenotype and antidepressant efficacy and not necessarily between genotype-

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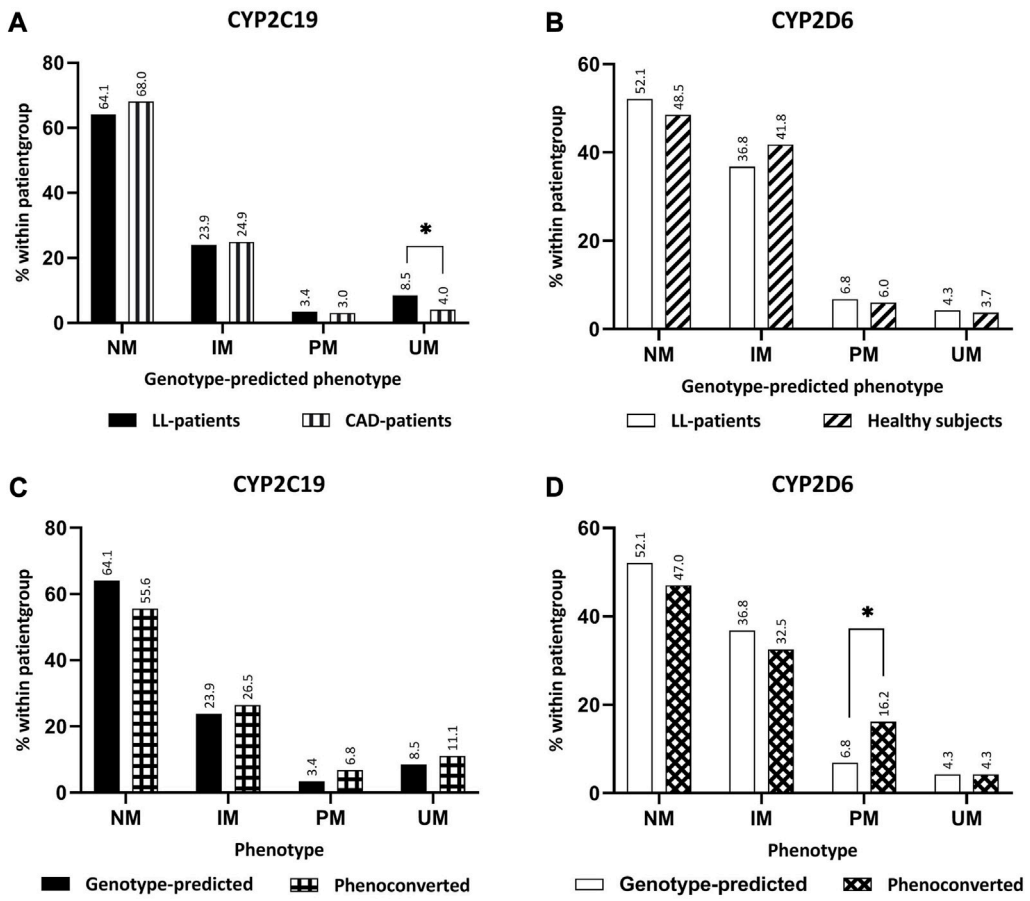

FIGURE 2
Comparison between phenotypes. **(A)** Comparison genotype-predicted phenotype of *CYP2C19* between LL-patients (n = 117) and CAD-patients (n = 820). **(B)** Comparison genotype-predicted phenotype of CYP2D6 between LL-patients (n = 117) and healthy subjects (n = 134). **(C)** Comparison of genotype-predicted phenotypes versus phenoconverted phenotypes *CYP2C19*. **(D)** Comparison of genotype-predicted phenotypes versus phenoconverted phenotypes *CYP2D6*. Significant differences ($p < 0.05$) are depicted with an *. Abbreviations *NM*, normal metabolizer; *IM*, intermediate metabolizer; *PM*, poor metabolizer; *UM*, ultrarapid metabolizer; *LL-patients* patients from "Body and Life" outpatient clinic; *CAD-patients* Coronary artery disease patients.

predicted phenotype and the antidepressant efficacy (Gressier et al., 2015).

This study found that there are also some non-genetic factors that influence the UKU-score of psychiatric patients. Patients who have polypharmacy have an OR of 4.26 for a moderate or high total UKU-score and therefore experience more or more severe side effects. These results are consistent with other studies, the higher the number of drugs a patient uses, the higher the chance of side effects and drug-drug interactions (Maher et al., 2014; Malki and Pearson, 2020).

A non-psychotic diagnosis also seemed to have an influence on the amount of side effects experienced. LL-patients with a non-psychotic diagnosis had a crude OR of 2.60 compared to LL-patients with a psychotic diagnosis. However, this result was not statistically significant when it was corrected for other covariables. The relationship between diagnosis and side effects is not clear, because several other underlying factors can play a role. The diagnosis of patients not only tells us something about the psychiatric disease, but also about the possible pharmacotherapy with associated side effects. Moreover, patients enrolled in the LL-clinic typically have more complex and more persistent mental disorders and therefore the current pharmacotherapy may lead to more side effects.

BMI may also play a role in the amount and/or severity of the side effects experienced. Patients with a BMI > 40 had a median UKU-score of 23.5, which was significantly higher than patients with a BMI between 30 and 40 or lower than 30, who respectively had scores of 15 and 13. However, this result was not seen when looking at the association between the UKU-score and the BMI as the OR was not statistically significant. Patients with a higher BMI often have a different response to antidepressants or antipsychotics, most of the time needing higher doses and thus experiencing more side effects (Warrings et al., 2021). Obesity and psychiatric disease have a complex bidirectional relationship (Holt and Peveler, 2009; Woo et al., 2016). Therefore, it is important to also consider the weight effects of a certain drug when choosing effective treatment for the mental disorder (McElroy, 2009). These aspects are already incorporated into the LL-clinic at the UMC Utrecht (UMC Utrecht, 2022a).

TABLE 3 Comparisons of UKU-score with baseline characteristics and comparison of phenotypes CYP-substrate users and UKU-score.

Total population	Number (%)	Total score UKU (median [range])	*p*-value (*significant)
Total with known UKU	104 (88.9)	15.5 [0–52]	
Age, categorized			
43 or younger (ref)	52 (50.0)	16 [0–49]	
44 or older	52 (50.0)	13 [2–52]	NS
Gender			
Male (ref)	43 (41.3)	14 [2–52]	
Female	61 (58.7)	16 [0–51]	NS
BMI, categorized			0.064
< 30 (ref)	26 (25.2)	13 [2–51]	
30–40	51 (49.5)	15 [0–52]	NS
>40	26 (25.2)	23.5 [6–49]	0.126
Main diagnosis			
Psychotic (ref)	68 (65.4)	13 [0–46]	
Non-psychotic	36 (34.6)	22 [2–52]	**0.002***
Polypharmacy			
Yes	63 (60.5)	20 [4–52]	
No (ref)	41 (39.4)	11 [0–51]	**<0.001***
Only CYP-substrate users			
P-CYP2C19	15 (14.4)		NS
NM (ref)	9	16 [4–27]	
IM	3	28 [12–52]	0.115
PM	1	–	NS
UM	2	18 [16–20]	NS
P-CYP2D6	49 (47.1)		0.089
NM (ref)	21	14 [2–37]	
IM	16	19.5 [4–52]	0.145
PM	10	15 [6–46]	NS
UM	2	43.5 [36–51]	**0.029***
CYP2C19	15 (14.4)		NS
NM (ref)	11	16 [4–27]	
IM	3	28 [12–52]	0.101
UM	1	–	N.A.
CYP2D6	49 (47.1)		0.203
NM (ref)	25	18 [2–37]	
IM	19	16 [4–52]	NS
PM	3	12 [11–46]	NS
UM	2	43.5 [36–51]	**0.011***

Significant differences in *p*-values are depicted by an * and bold text. NS values have a *p*-value >0.2.
Abbreviations P-*CYP2C19* phenoconverted phenotype CYP2C19; *P-CYP2D6*, phenoconverted phenotype CYP2D6; *NM*, normal metabolizer; *IM*, intermediate metabolizer; *PM*, poor metabolizer; *UM*, ultrarapid metabolizer; *ref* reference.

TABLE 4 *Association between side effects with baseline characteristics and phenotypes of CYP-substrate users and UKU-score.*

Total population	Number (%)	Total score on UKU categorized			OR (95% CI)	
		Low (≤11, %)	Moderate (12–20, %)	High (≥22, %)	Crude	Adjusted
Total with known UKU	104 (88.9)	37 (31.6)	34 (29.1)	33 (28.2)	N.A.	N.A.
Age, categorized						
43 or younger (ref)	52 (50.0)	15 (28.8)	20 (38.5)	17 (32.7)		
44 or older	52 (50.0)	22 (42,3)	14 (26,9)	16 (30,8)	0.55 (0.25–1.25)	0.46 (0.18–1.19)
Gender						
Male (ref)	43 (41.3)	17 (39.5)	13 (30.2)	13 (30.2)		
Female	61 (58.7)	20 (32.8)	21 (34.4)	20 (32.8)	1.34 (0.60–3.02)	1.25 (0.51–3.08)
BMI, categorized						
< 30 (ref)	26 (25.2)	12 (46.2)	7 (26.9)	7 (26.9)		
30–40	51 (49.5)	19 (37.3)	21 (41.1)	11 (21.6)	1,44 (0.55–3.76)	1,42 (0.50–4.03)
>40	26 (25.2)	6 (23.1)	6 (23.1)	14 (53.8)	2,86 (0.87–9.43)	2,64 (0.72–9.64)
Main diagnosis						
Psychotic (ref)	68 (65,4)	29 (42,6)	24 (35,3)	15 (22,1)		
Non-psychotic	36 (34,6)	8 (22,2)	10 (27,8)	18 (50,0)	**2.60 (1,04–6.54)***	2,43 (0.90–6.55)
Polypharmacy						
Yes	63 (60.5)	14 (22.2)	22 (34.9)	27 (42.9)		
No (ref)	41 (39.4)	23 (56.1)	12 (29.3)	6 (14.6)	**4.47 (1.90–10.53)***	**4.26 (1.76–10.32)***
Only CYP-substrate users						
P-CYP2C19[a]	15 (14.4)					
NM + UM (ref)	11	4 (36.4)	4 (36.4)	3 (27.3)		
IM + PM	4	1 (25)	1 (25)	2 (50)	1.71 (0.13–22.51)	1.92 (0.09–49.40)
P-CYP2D6[a]	49 (47.1)					
NM + UM (ref)	23	9 (39.1)	5 (21.7)	9 (39.1)		
IM + PM	26	6 (23.1)	9 (34.6)	11 (42.3)	2.14 (0.62–7.39)	1.31 (0.30–5.68)
CYP2C19[a]	15 (14.4)					
NM + UM (ref)	12	5 (41.7)	4 (33.3)	3 (25.0)		
IM + PM	3	0 (0)	1 (33.3)	2 (66.7)	N.A.[b]	N.A.[b]
CYP2D6[a]	49 (47.1)					
NM + UM (ref)	27	9 (33.3)	6 (22.2)	12 (44.4)		
IM + PM	22	6 (27.3)	8 (36.4)	8 (36.4)	1.33 (0.39–4.58)	0.74 (0.17–3.30)

Significant differences in *p*-values are depicted by an * and bold text. NS values have a *p*-value >0.2.

Abbreviations *P-CYP2C19* phenoconverted phenotype CYP2C19; *P-CYP2D6* phenoconverted phenotype CYP2D6; *NM*, normal metabolizer; *IM*, intermediate metabolizer; *PM*, poor metabolizer; *UM*, ultrarapid metabolizer, ref reference, 95% *CI* 95% confidence interval.

[a]The different phenotype groups were combined because separately the sample size of the phenotype groups was too small to perform logistic regression.

[b]The number of patients with *CYP2C19* IM and PM is too small to perform logistic regression, therefore no result can be given for *CYP2C19*.

In this study it was also possible to look at the prevalence of CYP-specific side effects. It was found that 75.0% of the *P-CYP2C19* IMs and PMs experience nausea and vomiting compared to 9.1% of the NMs and UMs. Another study also found *CYP2C19* PMs have a higher chance of gastro-intestinal side effects when using an SSRI (Fabbri et al., 2018). Side effects that occurred more often in *P-CYP2D6* IMs and PMs were depression, increased dream activity and sleepiness. 64.0% *P-CYP2D6* IMs and PMs experienced the side effect depression compared to 30.4% of the NMs and UMs. The side effect depression is associated with antipsychotic use, possibly related to hyperprolactinemia (Milano et al., 2017). However, the relationship between hyperprolactinemia

TABLE 5 Comparison of specific CYP-related side-effects and phenotype of CYP2C19 in CYP2C19-substrate users. The side effects epileptic seizures, amenorrhea, galactorrhea and gynecomastia were not included because they were not present in any of the patients.

Item	Total % (n = 15)	CYP2C19[a]			P-CYP2C19[a]		
		NM + UM % (n = 12)	IM + PM % (n = 3)	p-value	NM + UM % (n = 11)	IM + PM % (n = 4)	p-value
Fatigue	60.0	58.3	66.7	NS	63.6	50.0	NS
Sleepiness	66.7	58.3	100	NS	54.5	100	NS
Depression	33.3	25.0	66.7	NS	27.3	50.0	NS
Tension/Inner unrest	46.7	41.7	66.7	NS	45.5	50.0	NS
Increased duration of sleep	20.0	16.7	33.3	NS	18.2	25.0	NS
Reduced duration of sleep	13.3	16.7	0	NS	9.1	25.0	NS
Increased dream activity	33.3	33.3	33.3	NS	36.4	25.0	NS
Paresthesia	6.7	0	33.3	0.200	0	25.0	NS
Increased salivation	26.7	33.3	0	NS	27.3	25.0	NS
Dry mouth	66.7	66.7	66.7	NS	72.7	50.0	NS
Nausea/vomiting	26.7	16.7	66.7	0.154	9.1	75.0	**0.033***
Diarrhea	13.3	16.7	0	NS	18.2	0	NS
Constipation	26.7	25.0	33.3	NS	27.3	25.0	NS
Micturition disturbances	13.3	8.3	33.3	NS	9.1	25.0	NS
Orthostatic dizziness	46.7	50	33.3	NS	54.5	25.0	NS
Palpitations/tachycardia	46.7	41.7	66.7	NS	45.5	50.0	NS
Increased tendency to sweating	20.0	16.7	33.3	NS	18.2	25.0	NS
Rash	46.7	41.7	66.7	NS	45.5	50.0	NS
Pruritus	33.3	33.3	33.3	NS	36.4	25.0	NS
Weight gain	26.7	25.0	33.3	NS	27.3	25.0	NS
Weight loss	13.3	16.7	0	NS	18.2	0	NS
Gynecomastia	6.7	8.3	0	NS	9.1	0	NS
Increased sexual desire	20.0	16.7	33.3	NS	18.2	25.0	NS
Diminished sexual desire	20.0	25.0	0	NS	27.3	0	NS
Erectile dysfunction	13.3	8.3	33.3	NS	9.1	35.0	NS
Ejaculatory dysfunction	6.7	8.3	0	NS	9.1	0	NS
Orgasmic dysfunction	40.0	41.7	33.3	NS	45.5	25.0	NS
Dry vagina	6.7	0	33.3	0.200	0	25.0	NS
Headache	13.3	8.3	33.3	NS	9.1	25.0	NS

Significant differences in p-values are depicted by an * and bold text. NS, values have a p-value >0.2.
Abbreviations *P-CYP2C19* phenoconverted phenotype CYP2C19; *NM*, normal metabolizer; *IM*, intermediate metabolizer; *PM*, poor metabolizer; *UM*, ultrarapid metabolizer.
[a]The different phenotype groups were combined because separately the sample size of the phenotype groups was too small.

and *CYP2D6* phenotype is unclear (Calafato et al., 2020). For the sleep-related side effects, some research indicates that these side effects are CYP-related, but different factors may influence this. For example, patients using a SSRI metabolized by CYP2D6 more often report nightmares as a side effect (Eugene, 2019). However, it also seems that psychiatric patients in general more often have more vivid dreams (Schredl and Schredl, 2018). The same goes for the relationship between sleep and psychiatric disorders (Krystal, 2012). Gynecomastia was a side effect more often seen in *P-CYP2D6* NM + UM group. However, this side effect occurred specifically by the two UM patients. These two patients had a very complex background, having multiple comorbidities and/or

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TABLE 6 Comparison of specific CYP-related side-effects and phenotype of CYP2D6 in CYP2D6-substrate users. The side effects epileptic seizures, amenorrhea and galactorrhea were not included because they were not present in any of the patients.

Item	Total % (*n* = 48)	CYP2D6[a]			P-CYP2D6[a]		
		NM + UM % (*n* = 27)	IM + PM % (*n* = 21)	*p*-value	NM + UM % (*n* = 23)	IM + PM % (*n* = 25)	*p*-value
Fatigue	66.7	66.7	66.7	NS	65.2	68.0	NS
Sleepiness	64.6	55.6	76.2	0.138	52,2	76.0	0.085
Depression	47.9	40.7	57.1	NS	30.4	64.0	**0.020***
Tension/Inner unrest	56.3	55.6	57.1	NS	47.8	64.0	NS
Increased duration of sleep	20.8	22.2	19.0	NS	21.7	20.0	NS
Reduced duration of sleep	22.9	25.9	19.0	NS	17.4	28.0	NS
Increased dream activity	35.4	33.3	38.1	NS	21.7	48.0	0.057
Paresthesia	18.8	25.9	9.5	0.149	26.1	12.0	NS
Increased salivation	22.9	22.2	23.8	NS	21.7	24.0	NS
Dry mouth	45.8	51.9	38.1	NS	47.8	44.0	NS
Nausea/vomiting	25.0	18.5	33.3	NS	13.0	36.0	0.067
Diarrhea	14.6	14.8	14.3	NS	17.4	12.0	NS
Constipation	16.7	11.1	23.8	NS	8.7	24.0	NS
Micturition disturbances	10.4	7.4	14.3	NS	8.7	12.0	NS
Orthostatic dizziness	43.8	48.1	38.1	NS	43.5	44.0	NS
Palpitations/tachycardia	41.7	37.0	47.6	NS	39.1	44.0	NS
Increased tendency to sweating	27.1	33.3	19.0	NS	34.8	20.0	NS
Rash	22.9	25.9	19.0	NS	30.4	16.0	NS
Pruritus	31.3	29.6	33.3	NS	34.8	28.0	NS
Weight gain	33.3	33.3	33.3	NS	34.8	32.0	NS
Weight loss	25.0	22.2	28.6	NS	17.4	32.0	NS
Menorrhagia	6.3	3.7	9.5	NS	4.3	8.0	NS
Gynecomastia	6.4	11.5	0	NS	13.6	0	0.095
Increased sexual desire	10.4	11.1	9.5	NS	13.0	8.0	NS
Diminished sexual desire	18.8	18.5	19.0	NS	21.7	16.0	NS
Erectile dysfunction	14.6	14.8	14.3	NS	17.4	12.0	NS
Ejaculatory dysfunction	6.3	3.7	9.5	NS	4.3	8.0	NS
Orgasmic dysfunction	22.9	18.5	28.6	NS	21.7	24.0	NS
Dry vagina	8.3	7.4	9.5	NS	8.7	8.0	NS
Headache	22.9	22.2	23.8	NS	17.4	28.0	NS

Significant differences in *p*-values are depicted by an * and bold text. NS, values have a *p*-value >0.2.
Abbreviations *P-CYP2D6*, phenoconverted phenotype CYP2D6; *NM*, normal metabolizer; *IM*, intermediate metabolizer; *PM*, poor metabolizer'; *UM*, ultrarapid metabolizer.
[a]The different phenotype groups were combined because separately the sample size of the phenotype groups was too small.

switching drugs when the questionnaire was filled out, and therefore it is unsure if the occurrence of gynecomastia is due to the phenotype of these patients.

Lastly, next to the UKU-results, there was also an analysis of the drug concentration in plasma, corrected for the dose and phenotype. A two times higher CD-ratio was seen for (*P-*)*CYP2D6* IMs and PMs,

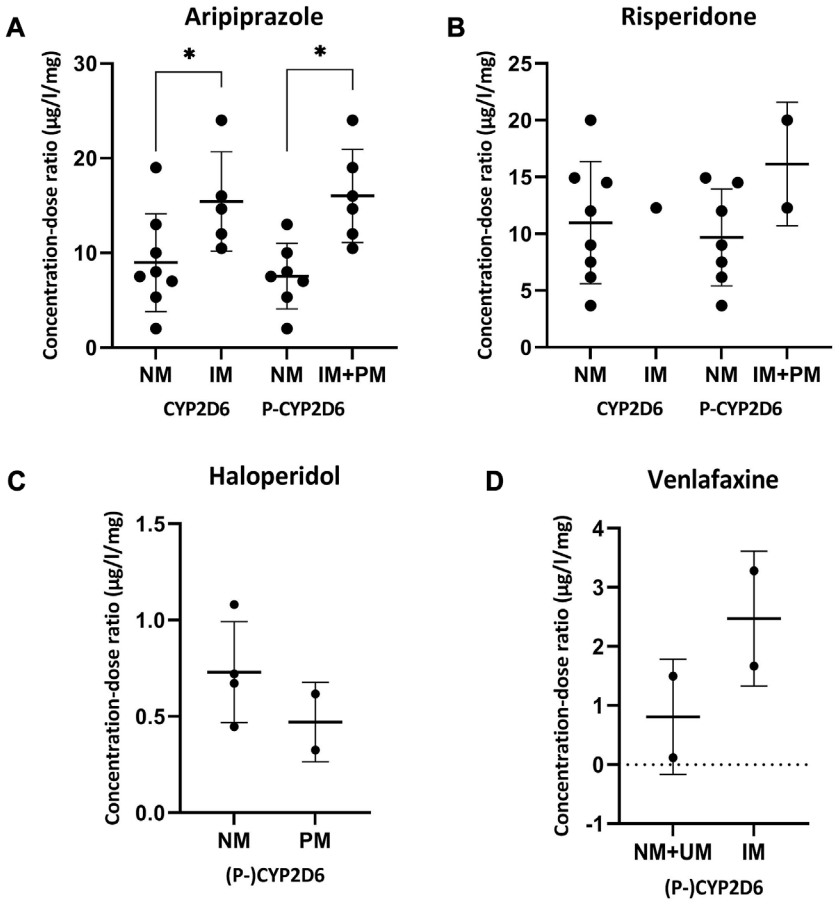

FIGURE 3
Concentration-dose ratios with SD of aripiprazole **(A)**, risperidone **(B)**, haloperidol **(C)** and venlafaxine **(D)** and the phenoconverted phenotype of CYP2D6. Significant differences ($p < 0.05$) are depicted with an *. The different phenotype groups were combined because separately the sample size of the phenotype groups was too small. For haloperidol and venlafaxine there was no difference in the patients in the different phenotype groups considering phenoconversion or not. Abbreviations *P-CYP2C19*, phenoconverted phenotype CYP2C19; *P-CYP2D6*, phenoconverted phenotype CYP2D6; *SD*, standard deviation; *NM*, normal metabolizer; *IM*, intermediate metabolizer; *PM*, poor metabolizer; *UM*, ultrarapid metabolizer.

which was statistically significant. These results are consistent with the findings of previous work (Jukic et al., 2019; Kiss et al., 2020).

This study has several strengths. First, general genotyping (i.e., without a specific reason) is not standard clinical practice for psychiatric patients. However, for each patient enrolled in the LL-clinic, a psychiatric PGx panel was performed. Moreover, this study considered phenoconversion, specifically by co-medication, and its effect on the phenotype. Most studies in the field currently focus on the genotype-predicted phenotype and do not take the effect of co-medication into account (Shah et al., 2016). Specifically in the psychiatric population there is a lot of drugs that are known CYP-modulators, so it is an important factor (Flockhart et al., 2022). For future research it is important to look at other factors that influence phenoconversion such as smoking, alcohol consumption and disease state (Klomp et al., 2020). Third, the UKU is a validated standardized questionnaire, specifically for psychiatric drug-users (Lingjærde et al., 1987).

The current study was, however, limited by the sample size. Only one patient per week was enrolled in the LL-clinic. This could be a limitation specifically for phenotypes like UM and PM because of their lower prevalence. Moreover, not every patient used a CYP-substrate drug and therefore not all patients could be included in the analysis of CYP-substrate users and phenotype and phenotype groups had to be combined for some of the analyses. To include enough PMs and UMs, large-sample prospective trials need to be conducted. Another limitation is the data collection. Genotypes and drug use had to be obtained manually. To minimize the chance of errors, this was done in a standardized manner. Lastly, extrapyramidal symptoms were not included in the adapted version of the UKU-questionnaire. Also, although side effects were collected through the validated UKU-questionnaire, some side effects may need to be objectified using laboratory tests or physical examinations (Lingjærde et al., 1987).

In conclusion, this study shows that phenoconversion is important to consider when looking at a patient's genotype. In the psychiatric population, where a difference in genotype distribution is observed, this phenomenon causes a shift from one phenotype to another. Although no significant associations were found between the phenotype and side effects experienced, there was a difference in the occurrence of specific side effects for the

different phenoconverted phenotypes. More research on this topic is important to take the next step towards better prediction of a patient's phenotype and possible prevention of side effects, contributing to personalized medicine.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Medical Research Ethics Committee Utrecht has been authorized (number 19-447/C). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: MdU, HH, WC, IW, and VD; Methodology: MdU, VD, and HA-K; Formal Analysis: MdU; Supervision: VD and HA-K; Preparing manuscript: MdU; Writing–first draft: MdU, HH, and VD; Writing–review and editing: MdU, HH, KW, HA-K, WC, IW, and VD. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1249164/full#supplementary-material

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Pharmacogenomics in clinical trials: an overview

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With the trend towards promoting personalised medicine (PM), the application of pharmacogenetics and pharmacogenomics (PGx) is of growing importance. For the purposes of clinical trials, the inclusion of PGx is an additional tool that should be considered for improving our knowledge about the effectiveness and safety of new drugs. A search of available clinical trials containing pharmacogenetic and PGx information was conducted on ClinicalTrials.gov. The results show there has been an increase in the number of trials containing PGx information since the 2000 s, with particular relevance in the areas of Oncology (28.43%) and Mental Health (10.66%). Most of the clinical trials focus on treatment as their primary purpose. In those clinical trials entries where the specific genes considered for study are detailed, the most frequently explored genes are *CYP2D6* (especially in Mental Health and Pain), *CYP2C9* (in Hematology), *CYP2C19* (in Cardiology and Mental Health) and *ABCB1* and *CYP3A5* (particularly prominent in Transplantation and Cardiology), among others. Researchers and clinicans should be trained in pharmacogenetics and PGx in order to be able to make a proper interpretation of this data, contributing to better prescribing decisions and an improvement in patients' care, which would lead to the performance of PM.

KEYWORDS

pharmacogenomics, pharmacogenetics, clinical trials, personalised medicine, clinical pharmacology, clinical research

1 Introduction

Personalised medicine (PM) was first defined in 2015 as a medical model using characterisation of individuals' phenotypes and genotypes (e.g., molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention. PM relates to the broader concept of patient-centred care, which takes into account that, in general, healthcare systems need to better respond to patient needs (European Union, 2023).

In this context, insights from pharmacogenetics and pharmacogenomics (PGx) become extremely useful to achieve such a level of personalised therapy.

While pharmacogenetics studies the individual variations in drug response due to genetic causes (Motulsky, 1957; Evans and Ckarke, 1961; Nebert, 1999), PGx is a broader based term that encompasses the simultaneous impact of multiple mutations in the genome that may determine a person response to drugs (Dere and Suto, 2009; Adams, 2008).

Although it is noticeable that drug data sheets now contain information on pharmacogenetic and PGx recommendations, not all the regulatory agencies have implemented this type of information to the same extent. The Pharmacogenomics Knowledge Base (PharmGKB) (Pharmacogenomics Knowledge Base, 2023; Whirl-Carrillo et al., 2021; Whirl-Carrillo et al., 2012), under its "Drug Label Annotations" section provides pharmacogenetic information included in the summary of product

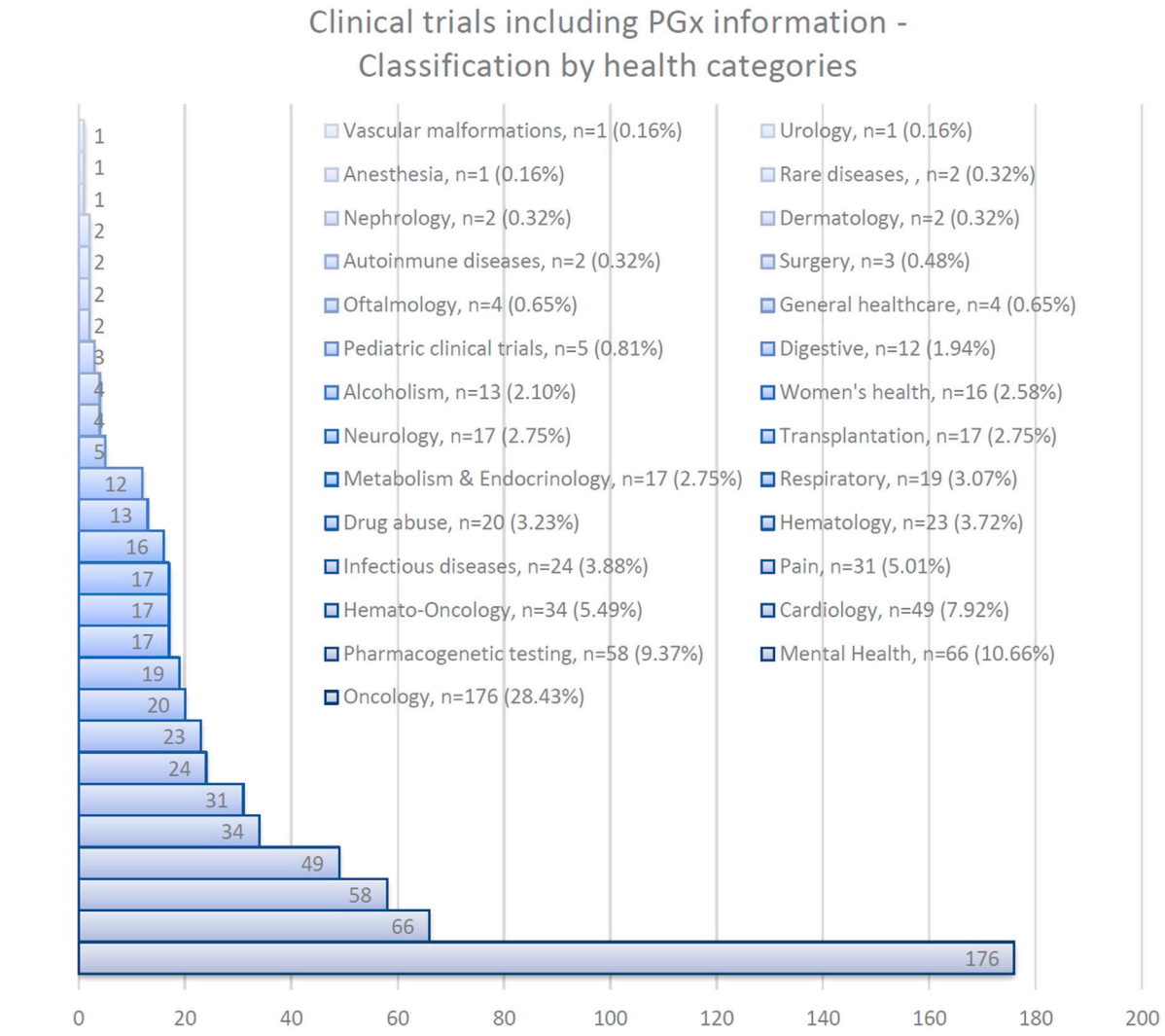

FIGURE 1
Clinical trials including PGx information classified by health categories.

characteristics of drugs approved by different regulatory agencies. See Supplementary Material.

There is a World Health Organization's publication from 2007 which already pointed out that PGx would cause significant changes in pharmacological research at the level of clinical trials conduct (Boulyjenkov et al., 2007).

Although the ethical challenges associated with the use of genetic information in clinical research should not be overlooked, the inclusion of PGx testing in clinical trials can help in the development of new medicines by contributing to a better understanding of their efficacy and safety (Pandya, 2017).

In this regard, clinical trials on medicinal products for human are a cornerstone for PM (Beccia et al., 2022; European Commission Directorate-General for Research and Innovation, 2011).

2 Materials and methods

A search on ClinicalTrials.gov (ClinicalTrials, 2023) was performed in order to review the clinical trials including PGx information available at the database. The terms "pharmacogenetics," "pharmacogenetics and PGx studies," "genetic" and "single-nucleotide polymorphisms" were included in the search field and the results were limited to interventional studies in order to obtain clinical trials information only.

The clinical trials were classified according to the health area they related to and their design.

Information about the clinical trial's location and characteristics from the enrolled participants and main genes studied were also obtained.

TABLE 1 Clinical trials including PGx information classified by design.

	Design characteristics	*N* = 619
Clinical Trial Phase	Early phase 1	4
	Phase 1	105
	Phase 1/Phase 2	13
	Phase 2	137
	Phase 2/Phase 3	23
	Phase 3	68
	Phase 4	127
	Not applicable	142
Clinical Trial Allocation	Randomized (*n* = 327)	Crossover assignment: 62
		Factorial assignment: 12
		Parallel assignment: 237
		Sequential assignment: 2
		Single-group assignment: 12
		No assignment information available: 2
	Non-Randomized (*n* = 107)	Crossover assignment: 1
		Parallel assignment: 53
		Single-group assignment: 47
		No information available: 6
	Not applicable	174
	No data	11
Clinical Trial Masking	None or Open Label	403
	Single (*n* = 37)	Investigator: 3
		Care provider: 1
		Participant: 17
		Outcomes assessor: 14
		Not described: 2
	Double (*n* = 65)	Participant and Investigator: 30
		Participant and Care provider: 8
		Care provider and Investigator: 1
		Investigator and Outcomes assessor: 6
		Participant and Outcomes assessor: 10
		Not described: 10
	Triple (*n* = 28)	Participant, Investigator and Outcomes assessor: 14
		Participant, Care provider and Investigator: 13
		Participant, Care Provider and Outcomes assessor: 1
	Quadruple (*n* = 71)	Participant, Care provider, Investigator and Outcomes Assessor: 71
	No data	15

TABLE 2 Most frequently studied genes in clinical trials including PGx information.

Gene	Number of CTs identified	Main health categories
CYP2D6	46 CTs	Mental Health, *n* = 13 (28.26%)
		Pain, *n* = 11 (13.91%)
CYP2C9	43 CTs	Hematology, *n* = 16 (37.21%)
CYP2C19	39 CTs	Cardiology, *n* = 11 (28.21%)
		Mental Health, *n* = 8 (20.51%)
ABCB1	39 CTs	Oncology, *n* = 11 (28.21%)
		Transplantation, *n* = 5 (12.82%)
		Cardiology, *n* = 5 (12.82%)
CYP3A5	34 CTs	Transplantation, *n* = 9 (26.47%)
		Oncology, *n* = 8 (23.53%)
CYP3A4	31 CTs	Oncology, *n* = 10 (32.26%)
		Pain, *n* = 5 (16.13%)
VKORC1	27 CTs	Hematology, *n* = 17 (62.96%)
UGT1A1	23 CTs	Oncology, *n* = 16 (69.57%)
COMT	19 CTs	Mental Health, *n* = 6 (31.58%)
		Pain, *n* = 4 (21.05%)
CYP2B6	17 CTs	Infectious diseases, *n* = 4 (23.53%)
SLCO1B1	15 CTs	Cardiology, *n* = 5 (33.33%)
OPRM1	12 CTs	Pain, *n* = 4 (33.33%)
DPYD	11 CTs	Oncology, *n* = 7 (63.64%)
CYP4F2	9 CTs	Hematology, *n* = 3 (33.33%)
		Cardiology, *n* = 2 (22.22%)

CTs, stands for clinical trials.

3 Results

The database search (from inception through 3 June 2023) returned 350,728 results of registered "interventional studies" (clinical trials). In the advanced search option, the "pharmacogenetics" term was added and 604 results of registered clinical trials that included "pharmacogenetics" or "pharmacogenetics and PGx studies" terms were obtained. To expand the scope and to ensure that those clinical trials that have not included those terms in their protocol description could be excluded, the terms "genetic" and "single-nucleotide polymorphisms" were included, obtaining 74 results more. After a review of the description of the purpose of these trials on a case-by-case basis and the elimination of duplicates, the final number of clinical trials with PGx-related information amounted to 619. Therefore, only 0.18% of the registered clinical trials contain these terms among the information provided for their inclusion in the system.

Review of the "Start Date" included in the database showed a considerable increase in the number of PGx-related clinical trials, especially from 2000 onwards. Thus, for example, while in 2000 there was only 1 trial with PGx-related information, this number increased to 4 in 2001, to 5 in 2002, to 12 in 2003, to 21 in 2004, to 31 in 2005. The year with the highest number of registered PGx-related clinical trials is 2010, with 53 trials.

With regard to PGx-related clinical trials, the areas of Oncology (28.43%) and Mental Health (10.66%), account for the largest number of trials registered.

Figure 1 provides a schematic overview about the clinical trials that include PGx information classified by health categories.

Regarding the recruitment status of these clinical trials, 376 of them are listed on ClinicalTrials.gov as completed (which means the study has ended normally and the last participant's last visit has been performed); 50 are terminated (the clinical trial has stopped early, will not start again and participants are no longer being examined or treated); 22 were withdrawn (the study stopped before enrolling its first participant); 37 are active, but not recruiting (the study is ongoing, but potential participants are not currently being enrolled); 10 are listed as not yet recruiting; 49 clinical trials are now recruiting; 2 are accessible to enroll by invitation; 2 were suspended; and there is no status information available about 71 of them.

On the reasons for the 2 clinical trials that figure as suspended, one informed that there were difficulties in recruiting patients and the other is waiting for the sponsor to raise funds for the remainder of the study.

In terms of participant characteristics, 542 clinical trials include both male and female participants, 49 trials include only women and 28 are male-only. With respect to subject's age, there were 42 clinical trials that include both child and adult participants, 546 clinical trials allow adult participants only (participants' age range has to be equal to or greater than 18 years) and 31 clinical trials aim at paediatric participants (under the age of 18).

For a description on the different clinical trials designs, see Table 1.

The information available at the ClinicalTrials.gov website was reviewed for each of the results to find out which genes or genetic variants were studied in each clinical trial. This revealed that in many cases this information had not been detailed to the registry. A large number of the studies mentioned the conduct of pharmacogenetic tests in the clinical trials in a broad manner, without specifying further. From the total number of registries, 274 (44.26%) report information indicating which genes or genetic variants are planned to be explored in the clinical trials. See Table 2 for information regarding the results observed on this point.

After obtaining this global information, for those health areas with the largest number of PGx -related clinical trials, the primary purpose defined on ClinicalTrials.gov was also consulted.

As shown in Table 3, the clinical trials' primary purpose is focused on treatment.

The countries with the highest number of PGx-related registered clinical trials are United States (*n* = 282, 45.56%), France (*n* = 57, 9.21%), Canada (*n* = 22, 3.55%), Netherlands (*n* = 19, 3.07%), Germany, United Kingdom (*n* = 18, 2.91% each one), Spain (*n* = 17, 2.75%) and the Republic of Korea (*n* = 16, 2.58%). It should be noted that 43 clinical trials (6.95%) are conducted at sites across different countries. There is no location data available for 13 registers (2.10%).

TABLE 3 PGx-related registered clinical trials' primary purpose.

	Clinical trial primary purpose								
	Basic Science	Health Services Research	Prevention	Screening	Diagnostic	Treatment	Supportive care	Other	No data
Oncology (n = 176)	1 (0.57%)	5 (2.84%)	6 (3.41%)	1 (0.57%)	4 (2.27%)	150 (85.23%)	4 (2.27%)	4 (2.27%)	1 (0.57%)
Mental Health (n = 66)	5 (7.58%)	2 (3.03%)	0	1 (1.52%)	4 (6.06%)	44 (66.66%)	6 (9.09%)	0	4 (6.06%)
Pharmacogenetic testing (n = 58)	9 (15.52%)	6 (10.34%)	2 (3.45%)	4 (6.90%)	4 (6.90%)	18 (31.03%)	4 (6.90%)	4 (6.90%)	7 (12.06%)
Cardiology (n = 49)	4 (8.16%)	2 (4.08%)	5 (10.20%)	1 (2.04%)	4 (8.16%)	29 (59.18%)	0	2 (4.08%)	2 (4.08%)
Hemato-oncology (n = 34)	1 (2.94%)	0	1 (2.94%)	0	1 (2.94%)	29 (85.29%)	0	2 (5.89%)	0
Pain (n = 31)	2 (6.45%)	0	0	1 (3.23%)	2 (6.45%)	22 (70.97%)	2 (6.45%)	2 (6.45%)	0
Infectious diseases (n = 24)	1 (4.17%)	1 (4.17%)	1 (4.17%)	0	0	19 (79.17%)	0	1 (4.17%)	1 (4.17%)
Hematology (n = 23)	0	0	2 (8.70%)	1 (4.35%)	2 (8.70%)	13 (56.52%)	1 (4.35%)	1 (4.35%)	3 (13.04%)

4 Discussion

PGx is still in the process of being incorporated into clinical trials. In this work, only 0.18% of the registered clinical trials contained any kind of PGx-related information. In general, the number of clinical trials including PGx information has been reported to be minimal (Burt and Dhillon, 2013).

In relation to the reported geographical distribution, the United States accounted for the greatest number of registered trials, which is consistent with the fact that this is also the region with the highest number of clinical trials overall, according to the data reported at WHO International Clinical Trials Registry Platform (ICTRP) (WHO ICTRP, 2023).

In this search, it was found that the area with the highest number of clinical trials with PGx information was Oncology. Within this therapeutic area, PGx plays a fundamental role, since in certain types of tumours, the knowledge of specific mutations can determine which treatment choice should be made (Filipski et al., 2014). In a review published by Sissung et al., the authors found that despite the large number of published clinical trials in Oncology (over ten-thousand phase I studies), fewer than 1% of these trials referred to the use of PGx in participants' stratification to optimise the design (Sissung and Figg, 2022).

Another therapeutic area where the incorporation of this type of information is particularly noteworthy is Mental Health. The development of clinical trials that include PGx information may help to improve our understanding about the mechanism of action of drugs used for the treatment of many psychiatric disorders (Pickar and Rubinow, 2001). A growing number of clinical trials with PGx information are appearing in the Mental Health field. As an example, Vos et al. published the results of a randomised clinical trial in patients with depression where the incorporation of pharmacogenetics-informed treatment (PIT) for tricyclic antidepressants was considered. The results showed that PIT allowed for therapeutic concentrations of tricyclic antidepressants to be reached earlier and also resulted in both fewer and less severe adverse effects (Vos et al., 2023). There is a systematic review and meta-analysis published in 2022 focused on examining prospective controlled clinical trials with PGx tests to assess the remission of depressive symptoms. The results of this work suggest a modest but significantly favorable effect of PGx-guided antidepressant therapy on depressive symptom remission (Brown et al., 2022).

Cardiology is another therapeutic area in which the number of reported clinical trials is particularly significant. Nevertheless, the translational of these Cardiology clinical trials' results into the clinic has been difficult for multiple issues, including the mixed results reported (McDonough, 2021) and also the different evidence support available depending of the specific pharmacological group evaluated (there are trials available regarding antiplatelet therapy, warfarin dosing, statin selection) (Duarte and Cavallari, 2021; Pereira et al., 2020; Claassens et al., 2019; Gage et al., 2018; Notarangelo et al., 2018; Bergmeijer et al., 2014; Voora et al., 2009).

When evaluating the incorporation of PGx from the very beginning of the drug development process, there are some benefits that should be mentioned. One of these benefits is an increased safety of clinical trials (Aneesh et al., 2009), as PGx may help to reduce patients' exposure to therapies that they have been identified in advance as not being responders to, or that may even be harmful to them (Gerogianni et al., 2018; Su et al., 2016; Plumpton et al., 2016; Franc, 2008; Ingelman-Sundberg, 2008). Another benefit is the reduction in drug development costs (Verbelen et al., 2017; Haycox et al., 2014; Aneesh et al., 2009) as well as in some time-related issues. It has been reported that including PGx in the early phases of clinical trials could contribute to reduce time both for the development process of a new medicine itself, as well as the time for its marketing (Pandya, 2017).

As interest in PM development is growing, it may be worth assessing how PGx information contributes at clinical trials' performing. This study attempts to provide an insight into pharmacogenetics and PGx's involvement in registered clinical trials.

Among the limitations of this study, it should be noted that there is not standarization in clinical trials' databases on how to reflect the type of contribution made by PGx to trials: patients' stratification, pharmacogenetic test to guide dosage, pharmacogenetic test to study

safety (adverse drug reactions), PGx-pharmacokinetics association study, therapy response assessment, etc. Therefore, more efforts should be made to propose improvements in this area.

There is currently an on-going guideline proposal, called STROPS (STrengthening the Reporting Of Pharmacogenetic Studies) that has integrated input from researchers, systematic reviewers and journal editors with the aim of improving the completeness and transparency of reports of PGx studies (Chaplin et al., 2020; Richardson et al., 2019; Strops, 2023).

It must also be noted that, besides the potential benefits associated to the incorporation of PGx to clinical trials, it is important to take into account that there is a parallel need to assess potential ethical risks when using genetic information in clinical research. The challenge is to strike a balance between the genetic information required for clinical trials without exposing participants to inappropriate use of their genetic data (Galende-Domínguez and Rivero-Lezcano, 2023). McKinnon et al. stated that ethical issues could be grouped into 3 categories: the equitable provision of healthcare, the possibility that genetic variants may track with race or ethnicity, and the questions of consent, access and privacy surrounding PGx information (McKinnon et al., 2007).

These ethical issues do not differ from those arising in other clinical circumstances (Gershon et al., 2014). For these reasons, it is recommended that genetic testing in clinical trials should be limited to the accomplishment of the main objectives stated in the approved protocol (Galende-Domínguez and Rivero-Lezcano, 2023).

In conclusion, it is certain that PGx should be integrated in clinical trials as a tool that can contribute to a better understanding about drugs efficacy and safety. Nevertheless, researchers and clinicians may not have sufficient PGx training as it has been previously reported (Behr et al., 2023; Kim et al., 2020; Chan et al., 2017), and this is a key point for them to be able to make a proper interpretation of all the PGx's data. There are now a number of pharmacogenetics and PGx information sources that clinicians and researchers should be familiar with and learn how to use, such as the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines (Clinical Pharmacogenetics Implementation Consortium, 2023; Caudle et al., 2014) or the very extensive information available at PharmGKB website.

Some proposals for improving training in this area include specific programmes for health science disciplines in faculties. In this sense, Gurwitz et al. published an article to enhance implementation of PGx and PM into core medical education and practice (Gurwitz et al., 2005). Different proposals have already been suggested by other authors over the last few years (Mosquera and Aleksunes, 2023; Haga et al., 2012; Pulley et al., 2012; Zgheib et al., 2011). Among them, as well as highlighting the importance of including specific programmes for health science disciplines already at the faculty level, it has been noted the importance of developing this knowledge at the residency. Some centres have already tested the implementation of PGx in their clinical practice and there are some publications reflecting the results (Caraballo et al., 2017).

For an assessment of the ethical aspects of PGx studies, there is a report from the Nuffield Council on Bioethics that can be consulted by health professionals and researchers (The Nuffield Council on Bioethics report, 2003; Corrigan, 2005).

All these efforts will contribute for a better drug prescribing and an improvement in patients' care, which by definition would lead to a PM for providing more individualised treatments.

Data availability statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Author contributions

RN-A designed the search, performed the analysis and wrote the draft of the manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Exploratory focused pharmacogenetic testing reveals novel markers associated with risperidone pharmacokinetics in Saudi children with autism

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Background: Autism spectrum disorders (ASDs) encompass a broad range of phenotypes characterized by diverse neurological alterations. Genomic studies have revealed considerable overlap between the molecular mechanisms implicated in the etiology of ASD and genes involved in the pharmacokinetic (PK) and pharmacodynamic (PD) pathways of antipsychotic drugs employed in ASD management. Given the conflicting data originating from candidate PK or PD gene association studies in diverse ethnogeographic ASD populations, dosage individualization based on "actionable" pharmacogenetic (PGx) markers has limited application in clinical practice. Additionally, off-label use of different antipsychotics is an ongoing practice, which is justified given the shortage of approved cures, despite the lack of satisfactory evidence for its safety according to precision medicine. This exploratory study aimed to identify PGx markers predictive of risperidone (RIS) exposure in autistic Saudi children.

Methods: This prospective cohort study enrolled 89 Saudi children with ASD treated with RIS-based antipsychotic therapy. Plasma levels of RIS and 9-OH-RIS were measured using a liquid chromatography–tandem mass spectrometry system. To enable focused exploratory testing, genotyping was performed with the Axiom PharmacoFocus Array, which included a collection of probe sets targeting PK/PD genes. A total of 720 PGx markers were included in the association analysis.

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; ASD, autism spectrum disorder; DOI, direction of impact; GWAS, genome-wide association study; HWE, Hardy–Weinberg equilibrium; IQR, interquartile range; LD, linkage disequilibrium; PD, pharmacodynamics; PGx, pharmacogenetic; PharmGKB, Pharmacogenomics Knowledge Base; PK, pharmacokinetics; QC, quality control; MAF, minor allele frequency; UM, ultrarapid metabolizer; NM, extensive or normal metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; RIS, risperidone; 9-OH-RIS, 9-hydroxyrisperidone; SNP, single-nucleotide polymorphism.

Results: A total of 27 PGx variants were found to have a prominent impact on various RIS PK parameters; most were not located within the genes involved in the classical RIS PK pathway. Specifically, 8 markers in 7 genes were identified as the PGx markers with the strongest impact on RIS levels ($p < 0.01$). Four PGx variants in 3 genes were strongly associated with 9-OH-RIS levels, while 5 markers in 5 different genes explained the interindividual variability in the total active moiety. Notably, 6 *CYP2D6* variants exhibited strong linkage disequilibrium; however, they significantly influenced only the metabolic ratio and had no considerable effects on the individual estimates of RIS, 9-OH-RIS, or the total active moiety. After correction for multiple testing, rs78998153 in *UGT2B17* (which is highly expressed in the brain) remained the most significant PGx marker positively adjusting the metabolic ratio. For the first time, certain human leukocyte antigen (HLA) markers were found to enhance various RIS exposure parameters, which reinforces the gut–brain axis theory of ASD etiology and its suggested inflammatory impacts on drug bioavailability through modulation of the brain, gastrointestinal tract and/or hepatic expression of metabolizing enzymes and transporters.

Conclusion: Our hypothesis-generating approach identified a broad spectrum of PGx markers that interactively influence RIS exposure in ASD children, which indicated the need for further validation in population PK modeling studies to define polygenic scores for antipsychotic efficacy and safety, which could facilitate personalized therapeutic decision-making in this complex neurodevelopmental condition.

KEYWORDS

exploratory, pharmacogenetic testing, autism, risperidone pharmacokinetics, array genotyping

1 Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by early onset in youth. However, its exact etiology, involving genetic and nongenetic (i.e., environmental) factors acting either alone or in combination, is still not clear. With the evolution in genomic technology and bioinformatics analysis techniques, several genetic mutations at both the gene and chromosome levels have been identified to be associated with different ASD phenotypes (Genovese and Butler, 2023). Recent reviews of ASD genomics using genome-wide association studies (GWASs) revealed considerable overlap between the molecular mechanisms implicated in the etiology of ASD and certain common genes involved in drug absorption, distribution, metabolism, and excretion (ADME) (Khanzada et al., 2017; Sundararajan et al., 2018; Fang et al., 2023). For example, 11 genes (*SLC6A3*, *UGT*, *GSK3B*, *HTR2A*, *MAOA*, *NOS1AP*, *PDE4B*, *TPH2*, *CACNA1C*, *CHRNA7* and *DRD2*) that influence serotonin and dopamine homeostasis and signal transduction pathways affecting mood, behavior and physical activity in ASD are also known to be associated with the pharmacokinetic (PK) and pharmacodynamic (PD) pathways of drugs employed in ASD management (Butler et al., 2016; Sundararajan et al., 2018; Sheng et al., 2021).

Therefore, it may be possible to observe variations in the PK and PD parameters of drugs in specific ASD patients when compared to other disease populations or normal volunteers (Genovese and Butler, 2020).

As a spectrum disorder, individuals with ASD usually exhibit variable degrees of behavioral and psychiatric manifestations, reflecting heterogeneity in the underlying etiology, which results in the segregation into different ASD phenotypes. This fact highlights the need for the implementation of precision medicine to enable individualization of psychopharmacological drug therapy regimens based on ASD subtype manifestations (Genovese and Butler, 2020). According to recent updates, the medications commonly used to address the comorbidities associated with ASD are atypical antipsychotics, which are frequently employed on a chronic basis according to standard dosing guidelines, which might not fit all etiological subtypes (Aishworiya et al., 2022; Ooi et al., 2023). Additionally, off-label antipsychotic use is still an ongoing clinical practice, despite the lack of evidence for its safety and tolerability (Højlund et al., 2021; Wang et al., 2021; Carthy et al., 2023). This practice was thought to be justified given the shortage of approved clinical cures and was even found to be motivated by advancements in diagnostic and clinician recognition of disparity in ASD and cooccurring mental health issues (Gupta and Gupta, 2023).

Risperidone (RIS) is a U.S. Food and Drug Administration (FDA)-approved atypical antipsychotic medication to target irritability often associated with autistic children (Lamy and Erickson, 2018). However, interindividual variability in RIS effectiveness and safety profiles has been reported in adults and children, even patients with similar diagnoses of psychiatric disorders, including ASD (Lamy and Erickson, 2018; Taurines et al., 2022; Liang et al., 2023). Moreover, RIS PK (exposure) parameters demonstrated wide interindividual variability within children with ASD (Dodsworth et al., 2018; Maruf et al., 2021).

RIS is mainly metabolized in the liver by the CYP450 isoenzyme CYP2D6; however, CYP3A4 and CYP3A5 have also been reported

to be partially involved in the 9-hydroxylation of RIS (Fang et al., 1999). 9-OH-RIS is a pharmacologically active metabolite that is approximately equipotent to the parent drug; therefore, both concentrations are collectively referred to as the total active moiety. 9-OH-RIS was later approved by itself as the antipsychotic paliperidone (Clarke et al., 2013). RIS and 9-OH-RIS efflux from cells are affected by certain transporter proteins, such as adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) (Yasui-Furukori et al., 2004; Saiz-Rodríguez et al., 2018).

Since the transformation of RIS to 9-OH-RIS is mainly mediated by CYP2D6, the ratio of the two molecules (RIS/9-OH-RIS ratio) in blood was classically suggested to be proportional to the CYP2D6 metabolic phenotype (Huang et al., 1993; Cho and Lee, 2006). Therefore, it is assumed that normal healthy subjects with a poor metabolizer (PM) status will have a higher metabolic ratio (less metabolic conversion of RIS) than extensive metabolizers (EMs; usually designated as normal metabolizers [NMs]) and ultrarapid metabolizers (UMs), as both conditions will result in a greater quantity of 9-OH-RIS (Huang et al., 1993; Cho and Lee, 2006; Novalbos et al., 2010). However, a large-scale study involving psychiatric patients of various ages revealed that the positive predictive value of an RIS/9-OH-RIS ratio >1 to predict *CYP2D6* PMs or <1 to predict *CYP2D6* UMs (95% CI) was 35% (26%–46%) and 9% (5%–14%), respectively (Mannheimer et al., 2016). Another pharmacogenetic clinical trial in healthy subjects demonstrated that CYP2D6 predicted only 65% of RIS metabolism variability and highlighted the demand for exploring pharmacogenetic predictors considering the complexity of its PK and PD pathway relationships (Gassó et al., 2014).

Collectively, these results indicated the presence of a potential research scope for examining other genetic markers of non-CYP2D6 variants (such as single-nucleotide polymorphisms (SNPs) involved in genes encoding transporters) (Yoo et al., 2011), which may affect RIS ADME and more comprehensively predict the extent of RIS and OH-RIS exposure (plasma levels) in healthy (Yoo et al., 2011) or unhealthy subjects, such as children with ASD (Troost et al., 2007; Sherwin et al., 2012; Roke et al., 2013; Youngster et al., 2014; Medhasi et al., 2016; Vanwong et al., 2016, 2017; Nuntamool et al., 2017; Rafaniello et al., 2017; Hongkaew et al., 2021; Kloosterboer et al., 2021).

Within the context of ASD, a systematic review of the current state of knowledge regarding CYP2D6 genetic variation and its impact on RIS PK and the propensity for adverse drug reactions in children and adolescents has suggested that CYP2D6 metabolic status was not consistently the sole genetic factor explaining the variabilities within these age groups (Dodsworth et al., 2018; Maruf et al., 2021). Despite the observed trend for a positive association between higher CYP2D6 activity and lower RIS concentration and RIS/9-OH-RIS ratios in some of the included studies (Troost et al., 2007; Youngster et al., 2014; Vanwong et al., 2016, 2017; Nuntamool et al., 2017), there was a consistent nonsignificant difference in the total active moiety concentrations among the different CYP2D6 phenotypes (PMs, EMs, and UMs) in all studies (Troost et al., 2007; Youngster et al., 2014; Vanwong et al., 2016; Nuntamool et al., 2017; Rafaniello et al., 2017; Vanwong et al., 2017). Additionally, these studies were conducted in ASD children of either Caucasian (Troost et al., 2007; Sherwin et al., 2012; Roke et al., 2013; Youngster et al., 2014; Rafaniello et al., 2017) or South Asian (Vanwong et al., 2016; Nuntamool et al., 2017; Vanwong et al., 2017) backgrounds, which could limit extrapolation of the results to other ethnogeographic groups (for example, Saudi Arabians) (McLellan et al., 1997; Al-Dosari et al., 2013) that may carry rare variants that are relatively less frequent in Europeans or South Asians.

Additionally, the few previous studies (Nuntamool et al., 2017; Rafaniello et al., 2017) that attempted to examine a limited number of other typical candidate pharmacogenetic (PGx) markers in RIS ADME (*ABCB1*, *ABCG2*, *CYP3A4*, *DRD2*, *DRD3*, and *HTR2A*) did not have a large enough sample size to make robust conclusions about a panel of genes or their variants or SNPs that should be included for individualized RIS therapy in ASD patients. Given the currently available conflicting data originating from candidate gene association study methods, "actionable" PGx markers related to antipsychotic dosing and selection, in general and with respect to different psychiatric conditions, have a limited application in routine clinical practice, even in developing countries, due to imperfect guidelines for interpretation and implementation (Eap, 2016; Eum et al., 2022).

Based on background information and consistent with the evolution of advanced technology, various pharmacogenomic approaches (targeted, focused or exploratory) can often be employed in human subjects to characterize the genetic determinants that may play roles in various aspects of drug activity and to support ongoing efforts to identify biomarkers predictive of drug exposure and/or safety in specific subgroups of diseases or certain age categories (Burczynski, 2009). Any of the three strategies can be pursued depending on the scale or number of genes in other ADME pathways that need to be examined in parallel (Table 1 in Burczynski, 2009). In our study, as dozens to thousands of genes are suspected to be involved in the drug metabolism or transport of RIS, both exploratory (nonhypothesis) and focused (guided) approaches were justified for identifying genetic alterations in all ADME genes (known PGx markers). Research with a multiplex genotyping approach is relatively innovative and is assumed to provide evidence for better optimum guidance of RIS dosing in physiologically and genetically modified settings, such as children with ASD, to avoid the risk of adverse drug reactions and/or suboptimal responses, as reported in several previous investigations (Correia et al., 2009; Nuntamool et al., 2017; Oshikoya et al., 2019; Shilbayeh S. A. R. et al., 2023).

Given the continuous growth in the knowledge base of DNA polymorphisms associated with ASD risk and ADME of antipsychotics, the current exploratory pharmacogenetic study was conducted with the aim of investigating potential PGx markers involved in RIS exposure (PK) in Saudi children with ASD and achieving a better understanding and clearer insights into the underlying mechanisms of disease-drug-gene interactions in this setting.

2 Materials and methods

2.1 Study design and participants

This study was a prospective cohort study conducted from November 2020 to February 2021 at three autism centers in

Riyadh, Saudi Arabia. All the methodological details, including screening, inclusion of candidate children, and data collection, were described previously in full detail (Shilbayeh S. A. R. et al., 2023).

Blood samples were collected for genotyping and RIS plasma level measurement as described previously (Shilbayeh S. A. R. et al., 2023).

2.2 Assay of plasma drug levels

RIS and 9-OH-RIS were extracted and measured in serum samples using a liquid chromatography tandem mass spectrometry system (LC/MS/MS, Waters, USA) according to a previously developed and validated method (Aravagiri and Marder, 2000). The lower limit of quantification of RIS and 9-OH-RIS was 1 ng/mL, and the lower limit of detection for serum RIS was 0.08 ng/mL and for serum 9-OH-RIS was 0.26 ng/mL.

2.3 Pharmacogenetic analysis

2.3.1 DNA extraction

Genomic DNA was extracted using the QIAsymphony^{SP} automated extraction system and a QIAsymphony$^{®}$ DSP DNA Midi Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). A Nanodrop spectrophotometer (Thermo Fisher Scientific, Santa Clara, CA, USA) was used to determine the concentration and purity of the extracted DNA.

2.3.2 Axiom PharmacoFocus Array

The Axiom PharmacoFocus Array (Catalog identifier: 952396; Thermo Fisher Scientific) offers comprehensive coverage of more than 2,000 markers (SNPs and insertions and deletions) in 150 genes across diverse populations (Tilleman et al., 2019) and functional variants that influence the ADME of commonly prescribed medications that are curated by the Pharmacogenomics Knowledge Base (PharmGKB) with clinical annotation levels of evidence 1A–2B (Whirl-Carrillo et al., 2021). These variants are commonly termed actionable PGx markers for testing in clinical practice (Whirl-Carrillo et al., 2021). Specifically, the Axiom PharmacoFocus Array facilitates genotyping in regions of high homology of key pharmacogenes (*CYP2A6*, *CYP2D6*, *GSTM1*, *GSTT1*, *UGT2B17*, and *SULT1A1*), which are usually difficult to obtain by complex multistep traditional methods (Tilleman et al., 2019).

According to the manufacturer's instructions, genomic DNA was amplified by multiplex polymerase chain reaction (PCR) using a QIAGEN Multiplex PCR Kit (Qiagen). These amplified products were then fragmented, pooled, resuspended, and hybridized to the PharmacoFocus Array platform (Thermo Fisher Scientific, Santa Clara, CA, USA). The array was scanned on the automated Applied Biosystems™ GeneTitan™ Multi-Channel (MC) Instrument (Affymetrix Inc., Santa Clara, CA, USA). The genotyping call rates and quality parameters of all available markers and samples were generated using Applied Biosystems Axiom™ Analysis Suite software (version 5.2, Thermo Fisher Scientific, Santa Clara, CA, USA).

To achieve the highest genotyping performance, samples that did not satisfy the Dish QC (DQC) parameters were excluded. Furthermore, individuals with a <90% genotyping call rate were excluded from the analyses. Moreover, all markers with any of the following criteria were not considered for the bioinformatic analyses: genotyping call rate <95%, minor allele frequency (MAF) < 0.05, Hardy–Weinberg equilibrium (HWE) $p < 0.001$, and located on the X chromosome.

2.4 Statistical and bioinformatic analyses

The identity by descent (IBD) test was performed using PLINK v1.9 (Purcell et al., 2007) to exclude samples with hidden relatedness. To calculate principal components (PCs), we used a total of 8319 probeset markers examined via the PharmacoFocus Array. Subsequently, variants were filtered according to the standard specified criteria: biallelic, passed aligner's QC, MAF >0.05, HWE $p > 0.001$, and no evidence of linkage disequilibrium (LD) ($r^2 < 0.2$). The remaining 4000 variants were used to perform PC analysis with PLINK v2.0 (Chang et al., 2015). Since 84% of the variation was explained by the first two PCs, they were used to remove ancestry and hidden relatedness biases in the association analysis (Supplementary Figure S1).

Association analysis was carried out with PLINK v2.0 (Chang et al., 2015). The 720 selected PGx markers were fitted into a generalized linear model with log2-transformed response values and adjusted by covariates including age, sex, PC1, PC2, and RIS medication history (duration, daily dose). The obtained *p* values were adjusted for multiple testing.

LD analysis was performed with PLINK v1.9 with an r^2 threshold of 0.5 and a window of 1,000 Kb. LD figures were generated for markers with significant associations (*p*-value <0.05) using HaploView v20.0.1 (Barrett et al., 2004), where blocks were defined by solid spine (SS).

3 Results

3.1 Study population

Of the 110 samples from pediatric patients with ASD who underwent clinical and psychological evaluations, 7 individual samples were excluded from genotyping for not meeting the DQC criteria, and 8 samples were excluded due to a call rate of <90% in the genotyping results. Furthermore, 6 patients who did not provide plasma samples for drug concentration determination were excluded. The average QC call rate for the passing samples was 99.2%.

Supplementary Table S1 displays the demographics and clinical criteria of the 89 patients in our study population. Eighty-three (93.3%) patients received RIS monotherapy. The majority of patients were male (N = 67, 75.3%), with a mean age of 9 (standard deviation (SD) = 4.1) years. The median RIS dose was 0.75 (interquartile range (IQR): 0.5–1.5) mg/day, with a median treatment duration of 21.5 (IQR: 3.23–57.9) months. Thirty-two (36%) patients also received concomitant psychotropic medications, primarily psychostimulants and melatonin. The median concentration of RIS was 0.56 ng/mL (IQR: 0.3–2.4) and that of 9-OH-RIS was 7.02 ng/mL (IQR: 2.4–13.4), while the active moiety concentration was 8.18 ng/mL (IQR: 2.8–16.4). The RIS/9-OH-RIS concentration ratio was 0.14 (IQR: 0.07–0.23).

TABLE 1 Top PGx markers associated with RIS plasma levels at a minimum $p < 0.01$.

Marker name	Associated gene	Chr.	rsID	MAF	OR (95% CI)	*p*-value	DOI
FDPS_c.-1-98T>G	FDPS	1	rs2297480	0.21	1.412 (1.149–1.737)	0.00166	+ve
ADRA2A_c.*427A>G/T	ADRA2A	10	rs553668	0.152	1.468 (1.154–1.867)	0.00260	+ve
TPMT_c.141-101A>T	TPMT	6	rs12529220	0.461	1.287 (1.093–1.514)	0.00341	+ve
TPMT_c.366 + 58T>C	TPMT	6	rs2518463	0.461	1.287 (1.093–1.514)	0.00341	+ve
HLA-DPB1:c.313A>G(Met105Val)	HLA-DPB1	6	rs1042151	0.225	1.334 (1.105–1.612)	0.00386	+ve
CYP2C19_41295G>A	CYP2C19	10	rs4494250	0.253	0.737 (0.604–0.9012)	0.0046	−ve
CYP2C18_c.*31C>T(3′UTR)	CYP2C18	10	rs2860840	0.253	0.737 (0.604–0.9012)	0.0046	−ve
NAT2_c.-594G>C(5′UTR)	NAT2	8	rs4271002	0.073	1.867 (1.224–2.848)	0.0057	+ve

Abbreviations: Chr., chromosome number; DOI, direction of impact; MAF, minor allele frequency.

3.2 Selection of PharmacoFocus PGx markers

A total of 8319 probeset markers were obtained via the PharmacoFocus Array, of which 2218 markers were identified as PGx markers. In the process of filtering 2218 PGx markers with the QC parameters, 100 (4.5%) markers were removed due to a global genotyping call rate of less than 95%. Of the remaining 2118 markers, 1,378 (62.13%) markers were excluded from further analysis because their MAF was less than 5%. Furthermore, 8 (0.4%) markers on the X chromosome and 10 (0.45%) markers with HWE *p* values <0.001 were omitted.

As a result, 722 (32.6%) of 2218 PGx markers were included in the association analysis. The average call rate of the 722 selected markers was 99.7%. The PCA plot did not show any clear clusters, indicating the absence of strong subpopulation stratification (Supplementary Figure S1).

3.3 Association of PGx variants with RIS PK parameters in the ASD cohort

A total of 27 PGx variants in 20 genes were demonstrated by PLINK software to have a significant association (*p*-value <0.01) with various RIS PK parameters measured in plasma, including RIS, its metabolite (9-OH-RIS), total active moiety, and RIS/9-OH-RIS metabolic ratio (results are displayed in Tables 1–4, respectively). The PGx markers are arranged in the tables according to their strength of association with the response variable. The direction of impact (DOI) of each individual PGx marker was positive or negative, indicating either increasing or decreasing an RIS PK measure, and is presented in its specific table.

Additional PGx markers that revealed potential associations with the RIS PK parameters with a minimum of $p < 0.05$ are presented in Supplementary Tables S2–S5. Nongenetic confounding variables, including age, sex, self-identified ethnicity, RIS dosage, treatment duration, and concomitant medications, were not significant in any of the models of RIS PK parameters.

First, out of all 722 included variants, only 8 markers in 7 genes were identified as top PGx markers for the RIS plasma level with $p < 0.01$ (Table 1; Figure 1A). Specifically, two SNPs (1 in *CYP2C19* (rs4494250) and 1 in *CYP2C18* (rs2860840)) were negatively correlated with the plasma level of RIS. However, the other top identified SNPs in five genes (*FDPS*, *ADRA2A*, *TPMT*, *HLA-DPB1*, and *NAT2*) were positively associated, indicating a greater effect on the RIS concentration estimates ($p < 0.01$). An additional 10 novel markers (i.e., not within the known RIS metabolic pathway) (Whirl-Carrillo et al., 2021) were identified to be associated at the level of $p < 0.05$, as shown in Supplementary Table S2. However, only one SNP (CYP2D6*4_1847G>A, splice site variant) in the *CYP2D6* gene, known to be primarily involved in the established RIS metabolic pathway (Whirl-Carrillo et al., 2021), was identified as significant at the level of $p < 0.05$ ($p = 0.04$). Although it had a low prevalence of 5% among the study sample, it was found to increase RIS levels by 1.5-fold (Supplementary Table S2).

Second, 4 PGx variants in 3 genes were found to be strongly associated with the level of the RIS metabolite (9-OH-RIS) at $p < 0.01$ (Table 2; Figure 1B). Interestingly, 2 of these SNPs are located in the *CYP2C8* gene, which encodes a novel CYP450 enzyme [CYP2C8] that was not previously known to influence the metabolism of RIS. The other 2 variants were identified in two different genes (*ABCC3* and *HLA-G*). However, at the level of $p < 0.05$, a total of 22 supplementary PGx markers in 17 genes were shown to have either a positive or negative effect on the metabolite concentration in plasma (Supplementary Table S3). Notably, among these secondary markers, 2 SNPs in the *CYP2C9* gene positively impacted the concentration of 9-OH-RIS by a 1.7-fold increase, reflecting the potential of the CYP2C9 enzyme to play an important role in the conversion of RIS to this metabolite.

Third, 5 markers in 5 different genes were shown to be significant in determining the total active moiety at the level of $p < 0.01$ (Table 3; Figure 1C). Only one PD marker (ADRA2A_c.*427A>G/T) was found to simultaneously enhance the exposure of RIS (Table 1) and the total active moiety at the level of $p < 0.01$ (Table 3). In the current study analyses, none of the metabolic PGx markers that affected RIS (Table 1) or 9-OH-RIS levels (Table 2) were simultaneously observed to have a significant impact on the total active moiety at the level of $p < 0.01$. This result indicated the possibility of their involvement in the conversion of RIS and 9-OH-RIS to other inactive metabolites.

Fourth, 10 SNPs in 5 genes were revealed to have a highly significant impact on the RIS/9-OH-RIS metabolic ratio (Table 4; Figure 1D). Notably, 6 SNPs (rs28360521, rs2267447, rs28588594, rs1080989, rs1065852, and rs3892097) in the *CYP2D6* gene

TABLE 2 Top PGx markers associated with 9-OH-RIS plasma levels at a minimum $p < 0.01$.

Marker name	Associated gene	Chr.	rsID	MAF	OR (95% CI)	*p*-value	DOI
CYP2C8*1B_-271C>A(5′UTR)	CYP2C8	10	rs7909236	0.101	0.3571 (0.1886–0.6762)	0.0023	−ve
ABCC3_c.3890G>A(R1297H)	ABCC3	17	rs11568591	0.0787	0.3196 (0.1502–0.6804)	0.0042	−ve
CYP2C8_1982A>G	CYP2C8	10	rs2275622	0.18	0.288 (0.156–0.532)	0.0052	−ve
HLA-G(rs66554220)	HLA-G	6	rs66554220	0.43	1.9811 (1.2277–3.1968)	0.0067	+ve

Abbreviations: Chr., chromosome number; DOI, direction of impact; MAF, minor allele frequency.

TABLE 3 Top PGx markers associated with total active moiety plasma levels at a minimum $p < 0.01$.

Marker name	Associated gene	Chr.	rsID	MAF	OR (95% CI)	*p*-value	DOI
ADRA2A_c.*427A>G/T	ADRA2A	10	rs553668	0.152	1.678 (1.233–2.283)	0.0016	+ve
CYP2E1*7B_c.-71G>T(5′UTR)	CYP2E1	10	rs6413420	0.157	0.611 (0.44–0.847)	0.0043	−ve
HLA-A(rs1061235)	HLA-A	6	rs1061235	0.121	1.532 (1.15–2.04)	0.0048	+ve
CRHR2(rs7793837)	CRHR2	7	rs7793837	0.368	1.454 (1.124–1.882)	0.0059	+ve
MTHFR_c.665C>T(Ala222Val)	MTHFR	1	rs1801133	0.129	0.602 (0.416–0.872)	0.0090	−ve

Abbreviations: Chr., chromosome number; DOI, direction of impact; MAF, minor allele frequency.

TABLE 4 Top PGx markers associated with the RIS/9-OH-RIS metabolic ratio at a minimum $p < 0.01$.

Marker name	Associated gene	Chr.	rsID	MAF	OR (95% CI)	*p*-value	DOI
UGT2B17_c.*317A>T(3′UTR)	UGT2B17	4	rs78998153	0.26	1.363 (1.185–1.568)	6.77×10^{-5}	+ve
CYP2D6_-2178G>A(5′UTR)	CYP2D6	22	rs28360521	0.13	1.447 (1.215–1.724)	0.0001	+ve
CYP2D6_2098A>G	CYP2D6	22	rs2267447	0.0734	1.705 (1.314–2.213)	0.0002	+ve
CYP2D6_-1426C>T(5′UTR)	CYP2D6	22	rs28588594	0.0734	1.705 (1.314–2.213)	0.0002	+ve
CYP2D6_-1000G>A(5′UTR)	CYP2D6	22	rs1080989	0.0734	1.705 (1.314–2.213)	0.0002	+ve
CYP2D6_100C>T(P34S)	CYP2D6	22	rs1065852	0.0734	1.705 (1.314–2.213)	0.0002	+ve
CYP2D6*4_1847G>A(SpliceDefect)	CYP2D6	22	rs3892097	0.0514	1.887 (1.342–2.652)	0.0006	+ve
HLA-DRB1(rs9272346)	HLA-DRB1	6	rs9272346	0.393	0.792 (0.679–0.925)	0.0046	−ve
CDA_c.435C>T(T145 =)	CDA	1	rs1048977	0.18	1.299 (1.074–1.571)	0.0091	+ve
CYP1A1_c.-27 + 606G>T	CYP1A1	15	rs2606345	0.309	1.251 (1.063–1.472)	0.0092	+ve

Abbreviations: Chr., chromosome number; DOI, direction of impact; MAF, minor allele frequency.

(preliminarily identified as the main metabolic enzyme guiding the conversion of RIS to 9-OH-RIS) were observed to significantly influence the metabolic ratio with an average OR = 1.7 (Table 4); no considerable effects related to these markers were found on the individual estimates of RIS, 9-OH-RIS, or the total active moiety plasma concentrations. Several other supplementary PGx variants were also shown to have an influence on the metabolic ratio but at a lower threshold ($p < 0.05$), as depicted in Supplementary Table S5.

3.4 Linkage disequilibrium analysis

LD analysis of the genetic markers that were associated with the 4 RIS PK parameters at the level of $p < 0.05$ is shown in Figure 2. Accordingly, on chromosome 1 (Figure 2A), *DPYD* rs2152878 and rs4492658 were observed to be in strong LD, while *FMO3* rs1736557 and *FMO1* rs12954 were noted to be likely in LD. On chromosome 2 (Figure 2B), *ABCB11* rs495714, rs473351, and rs497692 were found to be in strong LD. On chromosome 6 (Figure 2C), *TPMT* rs2518463 and rs12529220 were observed to be likely in LD; *HLA-A* rs1061235 and intergenic *HCG4* (rs1633021) and *HLA-G* (rs66554220) were noted to be likely in LD; *HLA-DQA1-AS* rs3129900, rs3129934, and rs9268542 and *HLA-DQA1* rs9272346 were in strong LD; *SLC22A1* rs1867351 and rs683369 were likely in LD; and *SLC22A1* rs683369, rs628031, and rs35854239 were in strong LD. On chromosome 10 (Figure 2D), *CYP2C9* rs4918758 and rs1505 and *CYP2C8* rs2275622 and rs7909236 were in strong LD and likely in LD with *CYP2C19* rs4917623. On chromosome 22 (Figure 2E), 6 *CYP2D6* SNPs (rs2267447, rs3892097, rs1065852, rs1080989, rs28588594, and rs28360521) were found to be in strong LD.

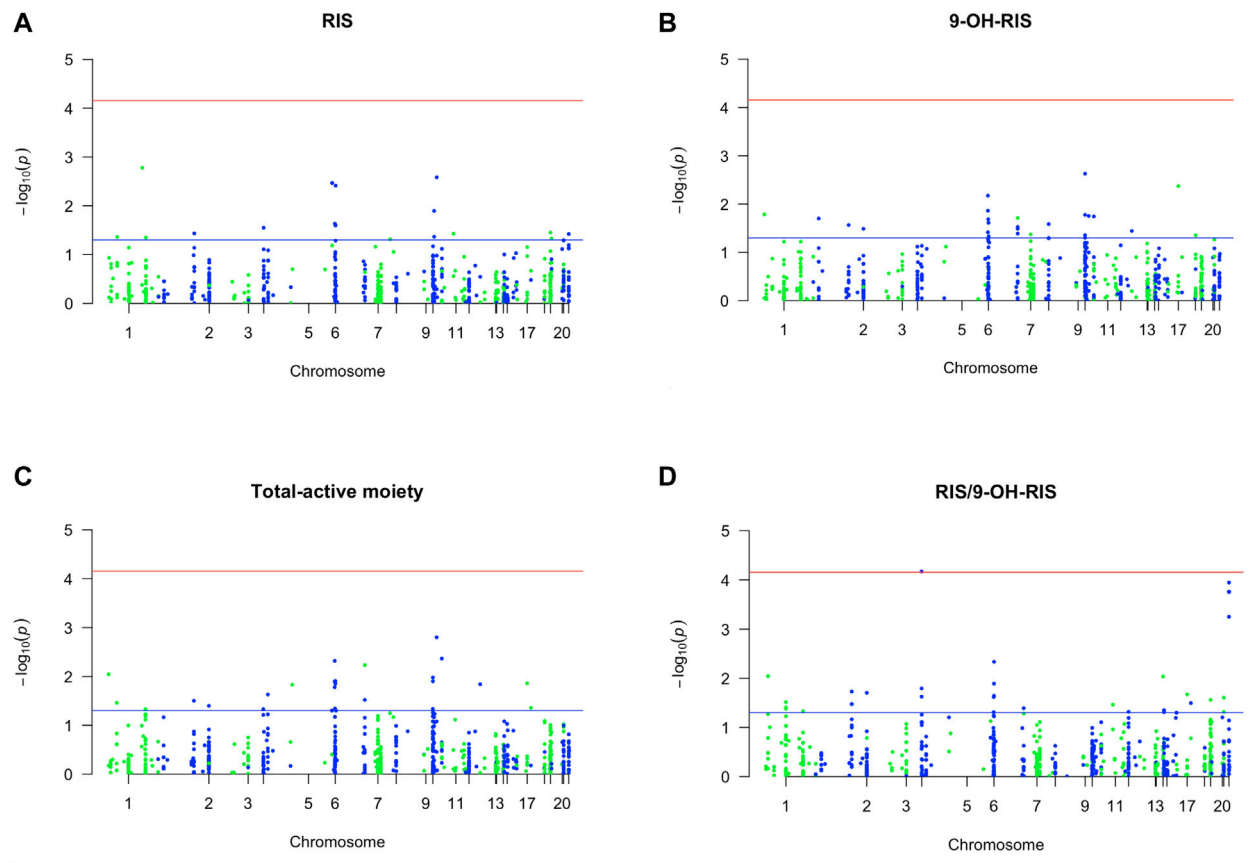

FIGURE 1
Manhattan plot of associations of RIS exposure parameters with 720 PGx PharmacoFocus markers. The horizontal x-axis represents the chromosomal position; the vertical y-axis represents $-\log_{10}$ P from the linear regression. The red horizontal line represents the significance level of $p = 7.0 \times 10^{-5}$ after Bonferroni correction. The horizontal blue line represents the significance level $p = 0.05$. **(A)** PGx variants of RIS exposure. **(B)** PGx variants of 9-OH-RIS. **(C)** PGx variants of the total active moiety. **(D)** PGx variants of metabolic ratio (RIS/9-OH-RIS).

4 Discussion

In this pharmacogenetic study, use of the Axiom PharmacoFocus Array platform revealed various novel PGx markers highly associated with RIS exposure in Saudi children with ASD. The key finding of this exploratory focused study is that most of the PGx markers that showed a prominent impact on various RIS PK parameters (27 out of 722 PGx variants examined) were not located within the genes involved in the classical RIS PK pathway, as previously defined by *in vivo* studies (Whirl-Carrillo et al., 2021).

4.1 PGx markers encoding phase I metabolic enzymes

4.1.1 CYP2C8 and CYP2C9

CYP2C8 is a phase I metabolizing enzyme that has been recently described by PharmGKB as a very important pseudogene (VIP) (Whirl-Carrillo et al., 2021). Interest in CYP2C8 emerged for several reasons, such as its central role in the biotransformation of structurally dissimilar compounds and endogenous molecules, attributed to its ability to bind divergent substrates without extensive conformational changes (Lai et al., 2009); its wide expression in body tissues other than hepatocytes (Sjostedt et al., 2020; Karlsson et al., 2021); more updates in identification of its substrates and inhibitors; and advanced knowledge in characterization of its SNPs and star alleles (Gaedigk et al., 2022). Additionally, the *CYP2C8* gene is positioned on chromosome 10q24 in the *CYP2C* gene cluster (centromere-*CYP2C18*-*CYP2C19*-*CYP2C9*-*CYP2C8*-telomere), and given the proximity of *CYP2C8* and *CYP2C9*, LD was previously reported between these genes (Yasar et al., 2002). Interestingly, the present exploratory study revealed strong LD between 2 *CYP2C8* (rs7909236 and rs2275622) and 2 *CYP2C9* (rs4918758 and rs1505) SNPs, all of which were shown to have significant individual associations with 9-OH-RIS plasma levels with decreasing (*CYP2C8* pair) and increasing impacts (*CYP2C9* pair). Only the *CYP2C8* pair was associated with a decreased total active moiety, possibly indicating increased metabolism of 9-OH-RIS. This assumption may be supported by evidence from previous studies indicating that rs7909236 (-271C>A SNP designated as *CYP2C8***1B*) is associated with normal enzyme function compared with wild-type (Bahadur et al., 2002; Yasar et al., 2002; Rodríguez-Antona et al., 2007). While this *CYP2C8* SNP was reported to be absent in Africans, its prevalence in our population (10%) was similar to that in Asians but lower than

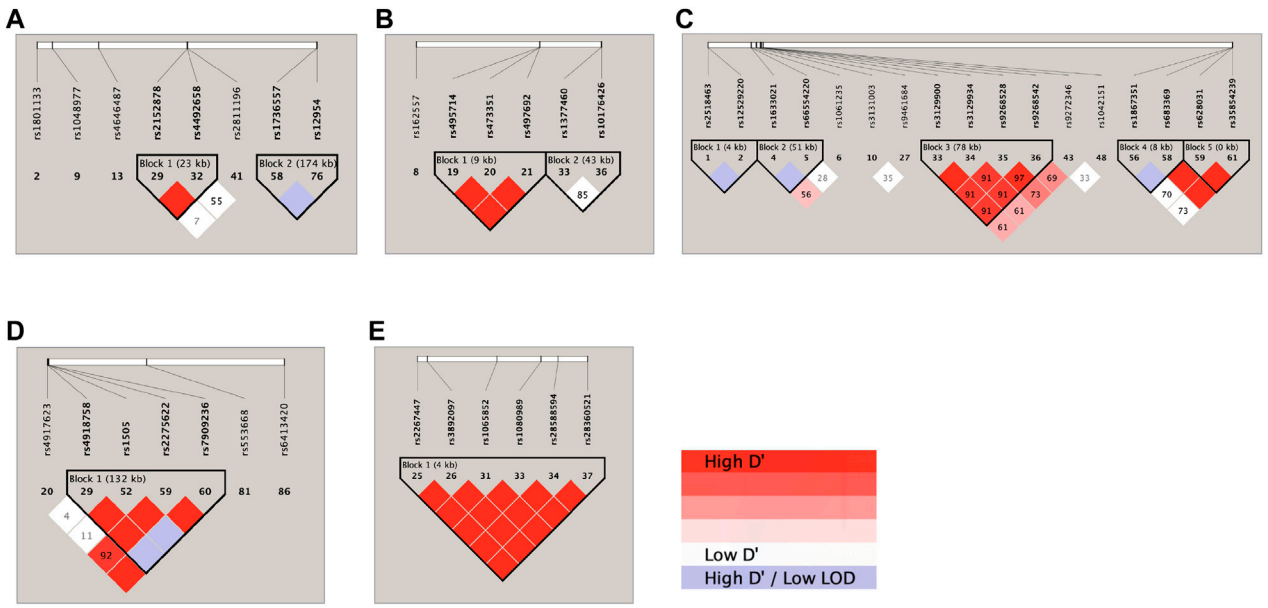

FIGURE 2
Haploview linkage disequilibrium map of SNPs associated with responses: **(A)** chromosome 1; **(B)** chromosome 2; **(C)** chromosome 6; **(D)** chromosome 10; and **(E)** chromosome 22. Pairwise linkage disequilibrium (D′) values are given in blocks for each SNP combination. Red indicates high D′ values; white indicates low D′; and violet indicates high D′ with a low logarithm of odds (LOD).

that in Caucasians (23%) (Bahadur et al., 2002). The other *CYP2C8* SNP (rs2275622) (18% in our population) is a variant that was less commonly reported in clinical studies with contradictory functional effects (associated with higher or lower enzymatic activity) depending on the substrate (Kirchheiner et al., 2008; Grau et al., 2009).

Collectively, the observed negative impact of CYP2C8 on 9-OH-RIS and the total active moiety support the assumption that this enzyme simultaneously acts on RIS and 9-OH-RIS, with a greater reduction in the RIS level (possibly highlighting the influence of CYP2C8 on a second RIS metabolic pathway to an alternative metabolite).

However, our observed increase in 9-OH-RIS plasma levels associated with the 2 *CYP2C9* variants (both designated as a *CYP2C9*1* allele with normal function) (Gaedigk et al., 2017) could be linked to increased RIS metabolism to 9-OH-RIS mediated by the CYP2C9 enzyme, presumably as a minor non-CYP2D6 pathway. In contrast, previous studies with smaller sample sizes have failed to reveal any CYP2C9 impacts on RIS PK parameters (Llerena et al., 2004; Cabaleiro et al., 2014).

Additional studies are needed to explain the relative contributions of CYP2C8 and CYP2C9 to RIS metabolism and the power of simultaneously existing *CYP2C8*/*CYP2C9* haplotypes to predict RIS efficacy and safety before implementation in clinical practice.

4.1.2 CYP2C19 and CYP2C18

Two SNPs from two genes encoding different CYP450 enzymes (1 in *CYP2C19* (rs4494250) and 1 in *CYP2C18* (rs2860840)) were observed to equally contribute to reduced concentrations of RIS among carriers compared to noncarriers. Unfortunately, the functional status of those two SNPs was not defined in the known resources of CYP2C19 or CYP2C18 Allele Definition Tables (Whirl-Carrillo et al., 2021; Gaedigk et al., 2022). However, both genes are within the CYP2C subfamily, which is responsible for the metabolism of various drugs, including warfarin, escitalopram and omeprazole; however, the role of CYP2C18 in drug metabolism in general remains unclear (Goldstein and de Morais, 1994). Interestingly, the two genes are closely located on the same chromosome, and previous evidence in Japanese subjects suggested complete linkages between the mutated alleles of *CYP2C18* and *CYP2C19* (Kubota et al., 1998). Furthermore, our observation of lower RIS concentrations among autistic children carrying rs2860840 (CYP2C18_c.*31C>T(3′UTR)) reinforces a recent novel finding by Bråten et al. (2021), indicating that increased CYP2C19-dependent escitalopram metabolism leads to decreased concentrations in *CYP2C19* NMs (*1/*1), similar to levels obtained in patients classified as *CYP2C19* UMs (*17/*17) or RMs (*1/*17). The latter findings were mainly attributed to the liver enhancer effect induced by only one SNP, *CYP2C18* (rs2860840), which was simultaneously carried by the *CYP2C19* NMs. This novel SNP finding was later examined in Native American cohorts, and due to its high frequency and clinical implications for the treatment of >20 drugs with official annotations for *CYP2C19* polymorphisms, it was recommended to add this SNP to the PGx practice panel to reveal the mismatching between *CYP2C19*-predicted and exposure-substantiated *CYP2C19* metabolic phenotypes (Fernandes et al., 2023).

Our study is the first to report on the significant individual and combined impact of two SNPs in *CYP2C19* and *CYP2C18* on reducing RIS plasma levels, which could lead to infectiveness and require a higher dosage to achieve the RIS target therapeutic range. In contrast, Cabaleiro et al. (2014) reported an increased chance of RIS-induced neurologic manifestations among *CYP2C19* NM healthy individuals

compared to *CYP2C19* PMs. However, in the same study, no significant impact of *CYP2C19* polymorphisms was found on RIS or 9-OH-RIS PK parameters. Whether this finding could be attributed to the absence of both SNPs identified in our study as enhancing the metabolism of RIS or the smaller sample size in the Cabaleiro et al. (2014) study remains to be examined in future large-scale studies.

4.1.3 CYP2D6

In the classical RIS metabolic pathway (Whirl-Carrillo et al., 2021), CYP2D6 is the primary enzyme involved in RIS metabolism to 9-OH-RIS. The current work reinforced this fact by revealing that 6 mutated *CYP2D6* PGx variants have a significant positive impact on the RIS/9-OH-RIS metabolic ratio, which indirectly reflects a substantial increase in the plasma bioavailability of RIS in comparison to its major metabolite. Notably, all these SNPs were in complete LD in our autistic children. However, none of these variants were shown to have an influence on the individual estimates of RIS, RIS metabolite, or total active moiety, which may reflect the presence of other, undiscovered non-*CYP2D6* variants (for example, as discussed earlier, *CYP2C8/9* haplotypes) that could further modulate global exposure to and the efficacy and safety of RIS therapy in ASD. This finding is consistent with a similar observation in an exploratory study in Thai children with ASD, which revealed that three *CYP2D6* variants existing in strong LD significantly influenced the metabolic ratio but not the discrete measurements of RIS, 9-OH-RIS, or total active moiety in plasma (Medhasi et al., 2016).

4.1.4 CYP2E1

CYP2E1 is a member of the CYP450 family that metabolizes relatively few prescription drugs but is better known for the metabolism of toxins and procarcinogens (Hayashi et al., 1991). Several *CYP2E1* SNPs in the promoter and intronic regions have been identified; however, luciferase promoter studies have shown that polymorphisms in the **7* haplotype, in particular the rs6413420 variant, increase *CYP2E1* transcription (Huang et al., 2012). Interestingly, the current study revealed a significant impact of this SNP (15.7% prevalence) in reducing the total active moiety (at the level of $p < 0.01$) and 9-OH-RIS ($p = 0.018$) up to 0.6- and 0.44-fold, respectively, compared to noncarriers. Consistent with this finding, two previous studies highlighted the role of *CYP2E1* SNPs in the etiology of schizophrenia and RIS treatment outcomes in the Chinese population (Huo et al., 2012; Shi et al., 2017). Of note, 5 *CYP2E1* SNPs (including **5*, rs3813867 and rs2031920) were associated with increased total active moiety, suggesting lower enzyme activity (Huo et al., 2012). However, rs2515641 in *CYP2E1* was found to be significantly related to nonresponse to RIS treatment for schizophrenia in a Chinese cohort ($p = 0.007$) (Shi et al., 2017). Additionally, molecular genetic studies found that DNA methylation levels of *CYP2E1* in the placenta were associated with children later diagnosed with ASD (Zhu et al., 2019; Bahado-Singh et al., 2022). Consistent with this finding, some molecular expression studies in inflammatory-mediated gastrointestinal diseases reported increased levels of CYP2E1 (122%) (Effinger et al., 2019), which strengthens the evidence of the role of the gut–brain axis in ASD etiology (Lombardi et al., 2023; Morton et al., 2023) and its correlation to our observation of reduced total active moiety and 9-OH-RIS levels in the study.

4.1.5 CYP1A1

The *CYP1A1* gene encodes the CYP1A1 enzyme, which is a member of the CYP1A subfamily and is responsible for the metabolism of diverse substrate molecules, such as sex hormones, caffeine, therapeutic drugs, environmental pollutants, toxins, and carcinogens. This diversity of CYP1A1 functions implies its involvement in numerous biological pathways (Kukal et al., 2023). Individual divergences in CYP1A1 expression and activity not only are attributed to genetic polymorphisms in *CYP1A* genes but can also be up- or downregulated through the interaction of environmental and endogenous physiological factors (Kukal et al., 2023). According to our association analysis, only one *CYP1A1* SNP (rs2606345), which was highly prevalent in our ASD population (30.9%), had a significant positive impact on the metabolic ratio. In PGx studies of other medications, this variant produced controversial findings regarding its influence on enzyme activity (Whirl-Carrillo et al., 2021). Despite the SNP's position in the intronic region of the *CYP1A1* gene, some PK studies reported decreased function (Allegra et al., 2016; Talwar et al., 2016), while others reported a gain of function (Grover et al., 2010; Cusato et al., 2014; Allegra et al., 2017). Therefore, it is quite challenging to interpret from current data the actual contribution of this *CYP1A1* polymorphism to the elevated metabolic ratio (increased RIS or decreased 9-OH-RIS levels in plasma), particularly because it is not directly involved in the primary RIS metabolic pathway. Moreover, this enzyme function was reported to be modified by a wide range of downstream modifications (genetic or epigenetic) and environmental factors that function together to alter the expression of the underlying genetic variant, leading to the ultimate biological response (Ye et al., 2019; Xu et al., 2023). Notably, another *CYP1A1* SNP (rs1048943) was highly prevalent in Thai children with ASD (30.3%) (Sukasem et al., 2016), indicating that further molecular studies are needed to define the mechanisms connecting the *CYP1A1* polymorphism to ASD and to modulate RIS PK in this disease population.

4.1.6 CDA

The *CDA* gene encodes the cytidine deaminase enzyme, which catalyzes the irreversible hydrolytic deamination of cytidine and deoxycytidine to uridine and deoxyuridine, respectively. It is one of numerous deaminases responsible for preserving the cellular pyrimidine pool. It is known that certain drugs can be rapidly metabolized by the CDA enzyme, which can affect their bioavailability and efficacy (Lavelle et al., 2012). In the context of chemotherapy, CDA plays a significant role (Abbaspour et al., 2023), since it metabolizes several chemotherapeutic drugs, including gemcitabine (Pellicer et al., 2017). Gemcitabine interferes with DNA synthesis and replication either by inhibiting enzymes involved in the synthesis of nucleic acid precursors or by misincorporation of nucleic acids into DNA or RNA macromolecules (Ciccolini et al., 2016). Studies have shown that the activity of the CDA enzyme can be a predictive biomarker in gemcitabine-treated cancer patients. Patients with lower CDA activity had significantly longer survival compared to patients with higher CDA activity (Soo et al., 2009). The current data suggested that one *CDA* variant (CDA_c.435C>T(T145 =), rs1048977) was a strong predictor for a higher RIS/9-OH-RIS metabolic ratio. However, to date, there is no specific

information in the medical literature about the interaction between CDA and RIS (Whirl-Carrillo et al., 2021). In the context of psychiatry and ASD, there is ongoing research into the role of various enzymes and their potential impact on these conditions. Some studies have demonstrated that *CDA* gene expression in the brain is associated with certain psychiatric disorders by creating DNA mutations via deamination of cytosine bases, which results in uracil (Gavin et al., 2012; Guidotti and Grayson, 2014). The same studies highlighted antipsychotics, including RIS, as potential targets in altering DNA methylation profiles in the brain. One study found that salivary immunoglobulin A (IgA) levels were significantly decreased in patients with ASD, and this correlated with bacteria-induced downregulation of the polymeric immunoglobulin receptor (Pigr) in salivary glands (Gong et al., 2021). However, this study did not specifically mention the CDA enzyme. Another study discussed the role of activation-induced CDA (AID) in the adaptive immune system and its potential implications in various diseases (Rios et al., 2020). However, it did not specifically link AID to autism or psychiatric disorders. While these studies provide valuable insights, more research is needed to fully understand the complex interactions among CDA, AID, and ASD etiology. Subsequently, it is important to investigate how genetic polymorphisms affecting *CDA* could act as markers for RIS clinical outcome (i.e., toxicity, efficacy) in real clinical practice.

4.2 PGx markers encoding phase II metabolic enzymes

4.2.1 FDPS

Another novel variant that was found to be associated with increased RIS levels was rs2297480 (by 1.4-fold, $p = 0.0012$) in the *FDPS* gene, which encodes farnesyl diphosphate synthase (FDPS), an essential enzyme in the mevalonate pathway (cholesterol biosynthesis) (Göbel et al., 2020). The *FDPS* gene is implicated in various diseases, including neuropsychiatric disorders such as ASDs (Segatto et al., 2019; Lin et al., 2023). Additionally, as an RNA-binding protein, FDPS is also involved in transcriptional and post-transcriptional regulation of many enzymes (Wang L. et al., 2023). A recent *in silico* analysis revealed that *FDPS* is an overlapping gene that is involved in CNS disorders and is simultaneously associated with the encoding of enzymes in the lipid and cholesterol metabolic pathways (Ang and Moon, 2022). Of note, antipsychotic drugs were observed to result in upregulated expression of the genes involved in cholesterol biosynthesis (Le Hellard et al., 2008), which was suggested as a potential causal pathway for their role in the pathogenesis of neuropsychiatric disorders (Zhou et al., 2021) as well as their subsequent induced adverse metabolic effects (Le Hellard et al., 2008). However, according to the present association study, how *FDPS* polymorphisms and expression modulate RIS exposure and response in ASD patients and *vice versa* remain unknown. However, our results highlight the need for further investigation of the pathways underlying this gene-disease-drug interaction.

4.2.2 TPMT

The *TPMT* gene encodes the thiopurine S-methyltransferase enzyme, which plays a crucial role in the metabolism of thiopurine drugs (Lee et al., 1995). Moreover, it is dependent on the S-adenosylmethionine (SAM) methyl donor substrate in the methionine pathway (Weinshilboum, 2006). The TPMT enzyme is involved with other conjugation enzymes in phase II detoxification, where liver cells add a substance (such as cysteine, glycine, methyl or a sulfur molecule) to a toxic chemical or drug to make it less harmful and easier for the body to excrete (Zhang, 2011). It has also been implicated in the metabolism of other aromatic and heterocyclic sulfhydryl compounds (Woodson and Weinshilboum, 1983).

Several pharmacogenetic studies have demonstrated that certain polymorphism-induced mutations in the *TPMT* gene result in completely undetectable TPMT enzyme activity, leading to life-threatening adverse events associated with even normal doses of anticancer drugs, such as azathioprine, cyclosporine, and daunorubicin (Wang et al., 2010). However, TPMT is not involved in the direct metabolic pathway (Phase I) of RIS (Whirl-Carrillo et al., 2021). In our study, two *TPMT* intronic SNPs (rs12529220 and rs2518463; both known to express the normal functional *TPMT* allele *1), with a prevalence of 46.1% among Saudi autistic children, were likely to be in LD at chromosome 6. Both were associated with a substantial increase in RIS levels (1.3-fold). Consistent with this finding, a previous RIS PK study in normal volunteers demonstrated significantly higher 9-OH-RIS plasma levels in *1/*1 genotype participants in comparison to mutant genotype carriers (*1/*2,*1/*3C,*1/*3A) (Cabaleiro et al., 2014). Another earlier study reported an association of decreased TPMT activity (mutant genotypes) with olanzapine-induced fatigue and dizziness in healthy volunteers with no significant impact on any of its PK parameters (Cabaleiro et al., 2013). However, no other data are available on their association with the PK of RIS or other antipsychotics (Whirl-Carrillo et al., 2021); therefore, it is challenging to provide a satisfactory interpretation. Further studies are needed to explore the impact of *TPMT* polymorphisms on chronic RIS therapy in ASD.

4.2.3 NAT2

The *NAT2* gene encodes N-acetyltransferase 2 (arylamine N-acetyltransferase), which is a typical xenobiotic metabolizing enzyme (Ackenheil and Weber, 2004) responsible for acetylation as a phase II conjugation reaction. In previous studies, NAT2 was identified to play a role in the metabolism of benzodiazepines (Camargo et al., 2023) and also hypothetically plays a role in the metabolism of some antipsychotics (Ackenheil and Weber, 2004). To date, 88 SNPs have been identified within the *NAT2* gene that can affect NAT2 function by resulting in reduced enzyme stability or altered affinity for a substrate. *NAT2* genotypes can be divided into three subgroups: “slow acetylator” (two slow alleles), “intermediate acetylator” (1 slow and 1 rapid allele), and “rapid acetylator” (2 rapid alleles, occasionally referred to as “fast”). Out of 38 *NAT2* SNPs examined in our exploratory study via the PharmacoFocus array, NAT2_c.-594G>C (5′UTR) (rs4271002) had a significant impact, increasing the RIS plasma level by 1.867-fold compared to noncarriers. Therefore, it is expected that carriers of this mutant allele may require dose modification to avoid RIS-induced adverse effects. Importantly, this is a novel *NAT2* variant that is not a part of any named alleles and has been shown in one study to be significantly associated with the risk of aspirin-intolerant asthma (Kim et al., 2010). According to PharmGKB, the functional consequence of this SNP is currently unknown, but it may affect

transcription, and its exact role in the RIS metabolic pathway remains unclear (McDonagh et al., 2014). However, our result is consistent with the impact of *NAT2* polymorphisms in the study by Cabaleiro et al. (2014) reporting an increased incidence of RIS-induced headache among *NAT2* IM and PM healthy individuals in comparison to *NAT2* NMs.

4.2.4 UGT2B17

The *UGT2B17* gene encodes uridine diphosphate glycosyltransferase 2 family, member B17, which is part of the family of UDP-glucuronosyltransferases (UGTs) (Beaulieu et al., 1996). As part of the phase II liver detoxification system, these genes are responsible for maintaining steady-state levels of a variety of substrates, including steroid hormones, by catalyzing the transfer of glucuronic acid moieties to these molecules and rendering them hydrophilic (Burchell et al., 1995). UGT2B17 is expressed not only in the small intestine and liver but also in steroid target tissues such as the breast, uterus, and prostate, where the extent of glucuronidation can be substantial (Karlsson et al., 2021), indicating its potential role in hormonally induced diseases (Wilson et al., 2004). However, molecular studies reported that UGT2B17 isoforms had a 4.4-fold higher abundance in the intestine than in the liver (Zhang et al., 2018). This fact suggested the potential of UGT2B17 to have a greater first-pass effect on its substrates when orally administered than subsequent liver metabolism, particularly in high UGT2B17-expressing individuals (Zhang et al., 2018). Additionally, in proteome studies, the UGT2B17 isoform, unlike other UGTs, was expressed in different brain regions, particularly in the cerebellum (Karlsson et al., 2021). According to the current study data, *UGT2B17* rs78998153, which had a prevalence of 26% among our ASD children, exhibited a very significant effect on RIS exposure. Indeed, after Bonferroni correction for multiple testing, this novel variant is the only PGx marker that still demonstrated a positive impact on the RIS/9-OH-RIS metabolic ratio, indicating a substantial increase in the RIS plasma circulating levels in comparison to its major metabolite. Consistently, an exploratory-based study of RIS PK in Thai children with ASD demonstrated that *UGT2B4* c.*448A>G (rs1131878), an isoform that is more highly expressed in liver than brain tissues (Karlsson et al., 2021), was highly associated with the metabolic ratio (Medhasi et al., 2016). Additional evidence for the impact of UGTs on RIS PK can be drawn from a study in Thai children with ASD, where three SNPs indicating *UGT1A1* mutation (an isoform that is highly expressed in brain tissues (Sjostedt et al., 2020)) have shown a significant association with the RIS-induced prolactin response (Hongkaew et al., 2018). Collectively, these results highlight the need for further molecular studies to explore the correlation between various *UGT* genotypes and their induced modifications in UGT enzyme expression in the brain tissues of patients with ASD. This research topic is anticipated to enable a better understanding of altered RIS disposition in the brain and its precise dose individualization requirements under this central nervous system condition (Sheng et al., 2021).

4.2.5 MTHFR

The *MTHFR* gene encodes the enzyme methylenetetrahydrofolate reductase (MTHER). MTHER is involved in a chemical reaction involving forms of the vitamin folate. Specifically, this enzyme converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This reaction is part of the multistep process that converts the amino acid homocysteine to another amino acid, methionine. The body uses methionine to make proteins and other important compounds, such as neurotransmitters (dopamine and serotonin) (Jalgaonkar et al., 2022; Majhi et al., 2023). Individuals homozygous for the SNP rs1801133 (MTHFR_c.665C>T(Ala222Val)) have lower *MTHFR* activity than CC or CT (heterozygous) individuals and therefore are predisposed to hyperhomocysteinemia associated with lower plasma folate levels (Majhi et al., 2023). A meta-analysis conducted on the association between *MTHFR* SNPs and ASD susceptibilities indicated that *MTHFR* rs1801133 was associated with ASD in the five genetic models (Li et al., 2020).

Consistent with this, a growing body of evidence suggests that the severity of autistic symptoms as a whole may be associated with increased levels of homocysteine associated with aggravation of dopamine deficiency (Carpita et al., 2023; Dangmann, 2023; Majhi et al., 2023). According to our data, rs1801133 of *MTHFR*, despite being less prevalent in our sample (12.9%) than in another cohort of Saudi children with ASD (36%) (Arab and Elhawary, 2019), was associated with a significant decrease in 9-OH-RIS and total active moiety levels, yet no evident impact was observed on RIS plasma exposure. The decreased concentration of the active moiety and a more pronounced effect on 9-OH-RIS could be explained by several factors. 9-OH-RIS undergoes minor hepatic metabolism and is primarily excreted unchanged by the kidney (79.6%). One of the known metabolic pathways for 9-OH-RIS is mediated by oxidative *N*-dealkylation, forming the acid metabolite M1 (Citrome, 2007; Vermeir et al., 2008). Emerging evidence indicates that high levels of homocysteine may enhance several metabolic pathways, such as oxidation (oxidative stress), nitrosylation, acylation, and hypomethylation (Perna et al., 2003a; 2003b). According to drug metabolism theories, these mechanisms (except hypomethylation) are believed to produce more polar metabolites that cannot diffuse across membranes and may, therefore, be actively transported (Li et al., 2019). Therefore, enhanced 9-OH-RIS excretion linked to hyperhomocysteinemia (induced by the MTHER mutation) via oxidation is assumed to be a superior postulated mechanism. In addition, homocysteine is a sulfur-containing amino acid (Lentz, 2005), which could serve as a co-factor in the conjugation of a drug metabolite by sulphation (Chen and Tang, 2022), leading to increased facilitated excretion in urine (Pan et al., 2020). However, these hypotheses remain uncertain. Clearly, more studies are necessary to elucidate the role of homocysteine in enhancing 9-OH-RIS excretion and its clinical consequences in ASD patients.

4.3 PGx markers encoding transporters

4.3.1 ABCC3

The *ABCC3* gene encodes a protein that is a member of the superfamily of ATP-binding cassette (ABC) transporters. These ABC proteins transport various molecules across extra- and intracellular membranes. The specific function of this transporter has not yet been determined; however, it was reported to mediate biliary and intestinal excretion of organic anions (Banach et al., 2022). The functional activity of some *ABCC3* variants has been fully examined (Singh et al., 2020). The current study revealed for the first time that a specific variant of the ABCC3 gene (ABCC3_

c.3890G>A(R1297H)) had a prominent negative impact on 9-OH-RIS plasma levels, indicating decreased excretion (efflux) from target cells (hepatocytes or brain) to the bile or peripheral blood. The negative impact of this variant on RIS clinical efficacy and safety in ASD carriers warrants further investigation.

4.3.2 ABCB11

The *ABCB11* gene encodes the ATP-binding cassette subfamily B member 11 protein, which is another member of the superfamily of ABC transporters. This membrane-associated protein is also named bile salt export pump (BSEP) or sister of P-glycoprotein (sPgp) (Strautnieks et al., 1998). Consistent with a previous exploratory study in Thai children with ASD (Medhasi et al., 2016), the current work detected the same 4 *ABCB11* variants (*ABCB11* rs495714, rs496550, rs473351 and rs497692), which displayed a significant reducing effect on the RIS/9-OH-RIS metabolic ratio, although at a lower rank of importance (Supplementary Table S4). Notably, our results in this population (Figure 2B) are compatible with the previous LD finding (Medhasi et al., 2016) that 3 *ABCB11* SNPs were strongly linked. This observation hypothetically indicates a more predominant influence of these mutations on reducing RIS efflux than 9-OH-RIS efflux, probably from various tissues' cells into bile or blood. In contrast, previous candidate gene studies in Caucasian children with ASD (Correia et al., 2009; Rafaniello et al., 2017) and adults with schizophrenia (Xing et al., 2006; Kuzman et al., 2008) have linked reduced RIS efflux with other ABC transporter subtype mutations (ABCG2_c.421C>A in *ABCG2*; c.3435C>T, c.1199G>A, c.1236C>T and c.2677G>T in *ABCB1*). These inconsistent findings emphasized the importance of genome-wide exploratory studies to reveal disease-specific PGx markers of certain drugs, with priority according to clinical significance and their ethnogeographic frequencies.

4.4 PGx markers encoding PD receptors

4.4.1 ADRA2A

One of the receptors that RIS blocks is the alpha 2A adrenoceptor (α2A-AR), which is an adrenergic receptor that responds to adrenaline and noradrenaline (Shahid et al., 2009). α2A-AR is encoded by the *ADRA2A* gene and is mainly found in the brain, where it regulates various functions, such as mood, cognition, attention, and sleep (Nyrönen et al., 2001). The role of α2A-AR in the therapeutic effects and side effects of RIS is not fully understood, but some studies have suggested that blocking this receptor may have both positive and negative consequences (Marcus et al., 2009). Additionally, other factors, such as genetic variations, drug interactions, and individual differences, may influence the response to RIS and the α2A-AR antagonism effect (Uys et al., 2017). The current association analysis revealed that *ADRA2A* SNP rs553668 carriers exhibit significantly increased RIS and total active moiety plasma levels (by 1.468- and 1.678-fold, respectively) in comparison to noncarriers. This finding could be interpreted in light of a previous study involving pheochromocytoma patients reporting that *ADRA2A* SNPs rs553668/rs521674 were associated with higher dosage requirements of α-adrenergic receptor blockers to control blood pressure (Berends et al., 2022).

Both our results and previous findings suggested that a certain degree of mutation in the α2A-AR receptor (decreased expression and/or density) mediated by these *ADRA2A* SNPs could lead to decreased drug-receptor occupancy and affinity, which could explain the subsequent higher PK exposure of any drugs targeted to antagonize it, such as that shown in our ASD patients exhibiting higher RIS plasma levels adjusted by the RIS dose. However, this assumption regarding the correlation between receptor affinity and drug plasma level remains speculative, and the functional consequences of the *ADRA2A* SNP on its receptor need to be examined. To our knowledge, to date, no other antipsychotic PGx studies have addressed the potential clinical consequences of polymorphisms of any of the genes encoding adrenergic receptors (including the *ADRA2A* gene) on efficacy and safety (Bousman et al., 2023). Therefore, it is important to investigate the current observation of significantly higher RIS plasma levels associated with *ADRA2A* polymorphisms in terms of clinical impacts in a larger cohort.

4.4.2 CRHR2

Corticotropin-releasing hormone receptor 2 (CRHR2) is a protein that is encoded by the *CRHR2* gene, which is highly expressed in the choroid plexus (part of the blood–brain barrier) of the human brain and to a lesser extent in the plasma membranes of hormone-sensitive cells, including those in the gastrointestinal tract and kidney (Pal et al., 2010). CRH is a hormone secreted from the hypothalamus in response to stress, which needs to efficiently bind with the CRHR2 receptor to stimulate its effects (Grammatopoulos et al., 1999). CRF is a key hormone that is involved in the control of various body systems via its mediatory stimulation of the hypothalamic–pituitary–adrenal (HPA) axis. On the other hand, hypothalamic CRF, via its action on the HPA axis, may be partially involved in the reinforcing effects of metabolic enzymes in phases I and II (Mormede et al., 2011; Wójcikowski and Daniel, 2011; Bromek and Daniel, 2021). In addition, recently, increased activation of the HPA axis was suggested to play an important role in ASD-like social behaviors (Jacobson, 2014; Rusch et al., 2023). As CRHR2 is one of the receptors for the hormones involved in the HPA axis, decreased CRHR2 expression levels in the hypothalamus were recently suggested to increase the risk of ASD (Wang X. et al., 2023). Interestingly, the current data revealed that an intronic *CRHR2* variant (rs7793837), which mostly causes mutations in the CRHR2 protein, was significantly associated with a positive impact on the total active moiety level in the plasma of children with ASD. These findings suggest that CRH could play a complex role in drug metabolism and possibly in the clinical response to RIS therapy, and further research could clarify its significance as a PGx marker within the context of ASD. A possible explanation for this increased level of RIS and 9-OH-RIS could be attributed to decreased binding of CRH to the mutated *CRHR2* variant 3 (rs7793837), leading to its decreased functional impact on various downstream signaling pathways mediating the metabolism and excretion of both molecules. However, this hypothesis needs to be proven in further studies to elucidate its clinical impact on RIS therapy outcomes.

4.5 PGx markers encoding immunity proteins

Growing evidence in recent decades has highlighted the role of alterations in immune function, including heightened inflammation,

anti-brain protein antibodies, and changes in T-cell and natural killer (NK) cell function in individuals diagnosed with ASD (Hsiao, 2013; Morton et al., 2023). Given that ASD may be induced by immunological or inflammatory pathological processes within the brain, GWASs have identified various associated human leukocyte antigen (HLA) risk alleles, including those related to *HLA-DPB1*, that obtained the most significant probabilities (Nudel et al., 2019; Morton et al., 2023). The current study revealed significant associations between *HLA-DPB1* (rs1042151), *HLA-G* (rs66554220), and *HLA-A* (rs1061235) and increased plasma levels of RIS, 9-OH-RIS, and total active moiety, respectively. A possible explanation for these influences could be attributed to the recently reported potential interplay between gut inflammatory processes mediated by these HLA markers and ASD incidence in children (the gut–brain axis) (Lombardi et al., 2023; Morton et al., 2023), thus leading to the postulation of pathophysiological changes in gastrointestinal permeability with subsequent alterations in RIS absorption and other possible inflammatory process-related consequences on the downregulation of the hepatic expression of its metabolizing enzymes or transporters (Effinger et al., 2019).

The latter hypothesis could be of interest for further examination in molecular expression studies involving ASD patients to confirm the impact of HLA-mediated inflammatory status on any medication's global PK, particularly those that are indicated for chronic use, such as RIS.

4.6 Strengths and limitations

Our study had several strengths and limitations. The first major strength of the current study compared with earlier candidate gene studies is the employment of an exploratory focused pharmacogenetic approach with a comprehensive array platform, which enables genotyping of most known PGx markers in genes related to the exposure and clinical consequences of most drugs, as curated by PharmGKB. Second, this is the first study to describe RIS PK in a cohort of Arabic children. To date, only a few RIS PK studies have been conducted, and these have focused mostly on children of European backgrounds (Maruf et al., 2021). Third, in addition to characterization of the exposure of RIS in Arabic children based on classical pathways, the modern methodology enabled identification of genetic variants of known association with ASD etiology that could specifically modulate RIS exposure. Fourth, our hypothesis-generating approach in this study revealed several novel PD SNPs that have a significant influence on RIS exposure in ASD children rather than PK SNPs alone, which indicated the need for further population PK modeling studies in this specific population (with more extensive blood sampling at several time points) to re-estimate the other RIS PK parameters such as steady-state volume of distribution and absorption and elimination rates. Modeling studies incorporating the PD, PK, and disease variants as predictors of interindividual variabilities in RIS plasma concentrations are speculated to enable more precise dosing and individualized therapy for children with ASD. Fifth, we employed LD analysis to reveal significant haplotype approximate loci that are not obtained by single genetic variation genotyping alone (Eberle et al., 2006).

However, there are also limitations that should be acknowledged. As detailed earlier, the study revealed significant associations for several PGx variants describing novel pathways (for example, immunity markers), but most of these associations did not remain significant after correction for multiple testing. This could be due to a lack of statistical power, in addition to the large number of markers tested in this study. However, our strict limitation to p values of less than 0.01 (together with 95% CI interval) in the top tier findings of this cohort has added confidence in adjusting for type I error to avoid false-positive findings. In addition, concerns were raised regarding the misuse and overly conservative practice of correction for multiple testing in exploratory studies due its potential to produce false-negative conclusions for significant markers that are certainly important (type II error) (Johnson et al., 2010). Alternatively, to ideally ensure richness in datasets' information to answer the exploratory research question (finding of innovative unanticipated associations), correction for multiple testing is statistically ill-advised, particularly if the modeling was adjusted by other justifiable techniques (such as PCs and nongenetic variables) (Rothman, 1990; García-Pérez, 2023). Second, since our study population has rarely been explored, our novel findings should be considered hypothesis generating and require validation in diverse ancestry cohorts. Third, in this study, RIS clinical outcomes reflecting effectiveness and safety were not examined; therefore, their relation to the novel variants identified in this study require further validation in future studies to confirm their utility in clinical practice with chronically treated patients with ASD. Finally, markers that failed the DQC parameters were removed from the present analysis. Some of these SNPs are related to the novel genes discovered in this study. Therefore, further association studies including these SNPs could strengthen the evidence of their related genes' impact on RIS PK.

5 Conclusion

In conclusion, the study provided strong evidence of an interplay of PK (metabolic enzymes), transporters, PD (receptors), and other novel groups of genetic variants (immune markers) in determining the RIS exposure level in ASD patients. The study also demonstrates the importance of an exploratory approach via the Axiom array technique, which has contributed to more precisely revealing and simulating the complex system of the pathophysiology of RIS disposition in children with ASD, in comparison to earlier candidate gene approach studies, where relevant genes were probably not fully addressed. Additionally, there could be physiologically relevant signaling pathways for some of our novel PGx markers that have not yet been revealed, and polymorphisms in genes influencing the signal transduction of these variants could also be of interest to reveal the complicated mechanisms underlying autistic phenotypes. Therefore, future studies in a larger cohort of diverse ancestry groups could confirm our current findings and improve the knowledge base on how these PGx variants could modify the efficacy of RIS or other antipsychotics and the risk of developing side effects in a broader range of ASD phenotypes, characterized by diverse neurological alterations, which could facilitate personalized therapeutic decision-making in this complex neurodevelopmental condition. In addition, the present findings could open a state-of-the-art track for mechanistic research into genetic informers of variability in antipsychotic exposure-mediated responses, which may indicate a novel approach for drug development.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by the Institutional Review Board (IRB) committees of both PNU (IRB Log Number: 20-0321) and KFMC (IRB Log Number: 20-758E). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

SS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing–original draft, Writing–review and editing. IA: Conceptualization, Data curation, Investigation, Validation, Writing–review and editing. EG: Methodology, Validation, Writing–review and editing. HA: Methodology, Writing–review and editing. KA: Methodology, Writing–review and editing. FA: Visualization, Writing–review and editing. AF: Data curation, Formal Analysis, Methodology, Validation, Visualization, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1356763/full#supplementary-material

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Assessing user perspectives on clinical pharmacogenomics consultation documentation: a user-centered evaluation

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The University of Florida Health Precision Medicine Program plays a crucial role in delivering pharmacogenomics (PGx) result notes to providers who request PGx testing. Despite this, there is currently a lack of a formal assessment of provider needs and established best practice design principles to guide the ongoing development of PGx result notes. This study aims to enhance the content and format of the PGx consult note at UF Health by incorporating valuable feedback from healthcare providers. Through in-depth user sessions involving 11 participants, we evaluated the usability of our consult note template. While overall satisfaction with the content was noted, specific sections, including those addressing phenoconversion and the medication list, were identified for revision to enhance clarity based on insightful provider feedback.

KEYWORDS

pharmacogenomics, consultation, user-centered evaluation, SOAP note, phenoconversion

1 Introduction

Established in 2011, the Precision Medicine Program (PMP) at the University of Florida Health (UF Health) initially focused on integrating pharmacogenomics (PGx) into routine clinical practice (Johnson et al., 2013). The program adopted a comprehensive system-wide approach, including the generation of written consult notes by PGx pharmacists (pharmacist with specialized training in PGx) for providers ordering PGx tests or requiring assistance with result interpretation. These consult notes, disseminated through the EPIC® electronic medical record (EMR) as clinical progress notes, have been instrumental in facilitating communication and collaboration.

The PMP PGx pharmacists utilize electronic means to inform ordering providers about the availability of consultation documents. Since its inception, the PMP clinical service has delivered 1970 consultation notes to 160 providers, catering to both clinical and research needs. Despite being designed for prescribers, no formal usability assessment has been conducted on these consult notes to enhance their effectiveness. Furthermore, guidance and literature reviews on PGx consult notes are limited, with the INGENIOUS trial (Eadon et al., 2016) (2016) being one of the few studies addressing concerns related to PGx consult notes. Notably, the trial highlighted issues such as information overload and the potential for

overwhelming providers with PGx information. Recognizing the necessity of a formal assessment, it is essential to leverage provider feedback to gauge satisfaction and the efficacy of documentation. Without such evaluation, the success of pharmacogenomic implementation may be impeded. Usability research, focusing on the user experience (UX) by deeply understanding users' needs, values, abilities, and limitations, emerges as a valuable tool to analyze provider feedback (Rosala; Yen and Bakken, 2011; Elchynski et al., 2021). This research can contribute to the enhancement of the entire PGx consultation process by informing the optimal content and format of PGx consult notes and fostering a better understanding of PGx. In pursuit of optimizing the usability of consult notes, our objective is to capture the perspective of provider needs, enhance the current content of PGx consult notes at UF Health, and guide future developments in PGx documentation.

2 Materials and methods

2.1 Study setting

The research was carried out at UF Health Shands Hospital, a substantial learning health system that utilizes EPIC® as its electronic medical record (EMR). The implementation of PGx consult documentation within the hospital is led by the UF Health Precision Medicine Program (Johnson et al., 2013), a team primarily comprised of PGx specialist pharmacists. All procedures were developed in alignment with the clinical consult notes used at the initiation of the study. Approval for the study was granted by the University of Florida Health Quality Improvement Project Registry.

2.2 Participant recruitment

Each participant was invited via email to participate in our in-depth user sessions (1-3 participants per session). We recruited UF Health clinicians who had previously ordered PGx testing and been the recipient of a PGx results interpretation (using a report generated from PGx clinical decision support alerts). Each session was moderated by either a PGx pharmacy resident or a pharmacy student and lasted between 30 and 60 min. Each participant verbally consented prior to the session through a secure online video chat.

2.3 Moderator guide development

A standardized moderation guide (see supplemental document A) was collaboratively developed by a team comprising two PGx residents, a PGx pharmacist, an informatics pharmacist, and two pharmacy students. The guide was designed for use with PGx consult notes from UF Health. In our study, we sought feedback on the logic of the consult note to help improve the content and format of PGx consultation. Our PGx clinical service typically employs the SOAP format (Subjective, Objective, Assessment, and Plan), a widely adopted format across healthcare systems (Pearce et al., 2016). We called this format "traditional format". In contrast, a flipped format, wherein the PGx test results and interpretation are positioned at the top of the note—an alternative format option preferred by the participants. At the time of study, both formats were implemented in clinical practice. Two sample notes (see supplemental document B) were incorporated into the moderation guide. Importantly, these notes were extracted from genuine patient PGx consult notes, ensuring compliance with HIPAA regulations by removing all patient identifiers. The flipped note illustrates a sample patient with a gastrointestinal case.

2.4 In-depth user sessions

Each sessions were led by 1-2 moderators and were recorded with the participants' prior consent. Before each session, participants were sent a modified Computer System Usability Questionnaire (CSUQ) (Lewis, 2018), a validated computer usability satisfaction questionnaire via REDCap® survey (UF Redcap, Nashville, TN). The CSUQ survey sought clinicians' assessments of the current PGx consult note across various characteristics. We combined and presented data in seven categories: organization, ease of comprehension, information quality, clarity of the future medication and phenoconversion section, helpfulness, and overall satisfaction. We modified the questionnaire to replace "this system" by "this note" or "phenoconversion session" to improve the clarity of survey questions. Providers were presented with a set of statements and asked to express their opinions on each, ranging from strongly disagree to agree for each statement (Lewis, 2018). Additionally, the survey gathered information about participant demographics, their experience with EPIC® EHR, and reflections on their knowledge of PGx.

During the sessions, participants were introduced to a set of two sample PGx consult notes, representing different clinical scenarios while adhering to the existing content and format. They were given a few minutes to familiarize themselves with each consult note. The moderator then initiated a series of predetermined and impromptu questions through a standardized script (see supplemental document A) to assess the participants' ease of comprehension and application of pharmacogenomic information. The questions also delved into the appropriateness of specific sections' presence and placement, such as relevant laboratory markers and the patient's past medication list. This approach was employed to ensure consistency across sessions and maintain a structured exploration of participants' perspectives.

2.5 Data collection and outcome measures

Data from the in-depth user sessions were captured using Zoom® (2022 version, San Jose CA) video and audio recordings. Zoom® video recordings allow for both the participant and the moderator's monitor display to be recorded. The recordings were then transcribed using the transcribing software Grain® (2021 version, San Francisco CA) and reviewed independently by two analysts (ND and NR) to extract suitable content for analysis. Three analysts (ND, BH, NR) and one pharmacogenomic specialist (EE) analyzed the first three sessions and codified the data to

TABLE 1 Main description of each section used for analysis.

Sections	Description
Subjective/Objective	Encompasses statements related to various sections, including the History of present illness, current medications affected by pharmacogenomic results, relevant pharmacogenetic test results, relevant labs, relevant drug interactions, and phenoconversion. Also includes statements about the outpatient EPIC-generated medication list and drug allergies
Phenoconversion	Involves statements regarding the overall clarity and ease of use of the "Clinical Phenotype" section of the note, along with its interpretation
Assessment	Encompasses statements regarding the "Test Results Interpretation" section within the Assessment section of the progress note
Plan	Involves statements made in regards to the "Plan" section of the note, including any feedback or observations related to this aspect
Flipped Note	Encompasses statements referring to a format of the progress note where the "Plan" section is positioned at the top of the note. Focuses on user feedback and perceptions of this format
PGx Table	Encompasses statements related to the PGx table found in the "Plan" section of the note. Feedback or comments specific to this table are included in this category
General	Encompasses statements that are nonspecific to a particular section. Includes overall recommendations or general feedback related to the entire note

TABLE 2 Participant Demographics. Characteristics of the ten providers who participated in the in-depth user sessions.

Participant characteristics	Results *n* = 10, (%)
Female Sex (%)	3 (30)
Years of Practice (%)	
5–9	1 (10)
10–14	3 (30)
15–19	2 (20)
20 or more	2 (20)
Self-Perception of PGx Knowledge (%)	
Have some idea with PGx, however, does not know how to apply the information	2 (20)
Clear idea, however have not used PGx in practice	1 (10)
Can explain the concept of PGx, and is comfortable using it in their practice	7 (70)

[a]Demographic data of one participant were corrupted and cannot be analyzed.

establish common themes, utilizing the qualitative data analysis software Nvivo® (v11 plus, Denver CO). Our thematic analysis focused on specific sections of the consultation note (subjective, phenoconversion, assessment, plan, PGx table, flipped note concept, and general idea), reflecting the structure of the in-depth sessions. Table 1 provides definitions for each section. We evaluated each section based on strengths, weaknesses, and suggestions for improvement. After establishing common themes, multiple analysts independently reviewed each session. Disputes were resolved by a team of four analysts and a PGx specialist. To ensure rigor, at least three individuals reviewed each session. Finally, CSUQ data were presented quantitatively as mean, median and interquartile range.

3 Results

Table 2 displays the demographics of the study participants as well as their initial assessment with PGx knowlege. We sent out invitations to 79 potential participants between January 2022 and February 2022. Eleven providers were recruited and interviewed for the study (response rate 15%), but only 10 participants were included in the demographics analysis due to a data error (unretrievable) in one participant's information. Data from this participant was still included by the rest of the analysis. Among the participants, three providers had a practice experience ranging from 10 to 14 years, while two providers had practiced for 20 years or more.

Regarding participants' knowledge of PGx, the majority of providers (70%) expressed that they are comfortable applying their knowledge of PGx in their practice. Additionally, 20% stated that they had a conceptual understanding of the idea but faced challenges in applying the information.

The CSUQ survey findings revealed that four out of ten providers strongly agreed with the statement indicating satisfaction with the note's organization. On the other hand, six out of ten providers neither agreed nor disagreed regarding the clarity of the phenoconversion section, suggesting a potential opportunity for redesigning this specific section to enhance understanding and user experience (See Figure 1).

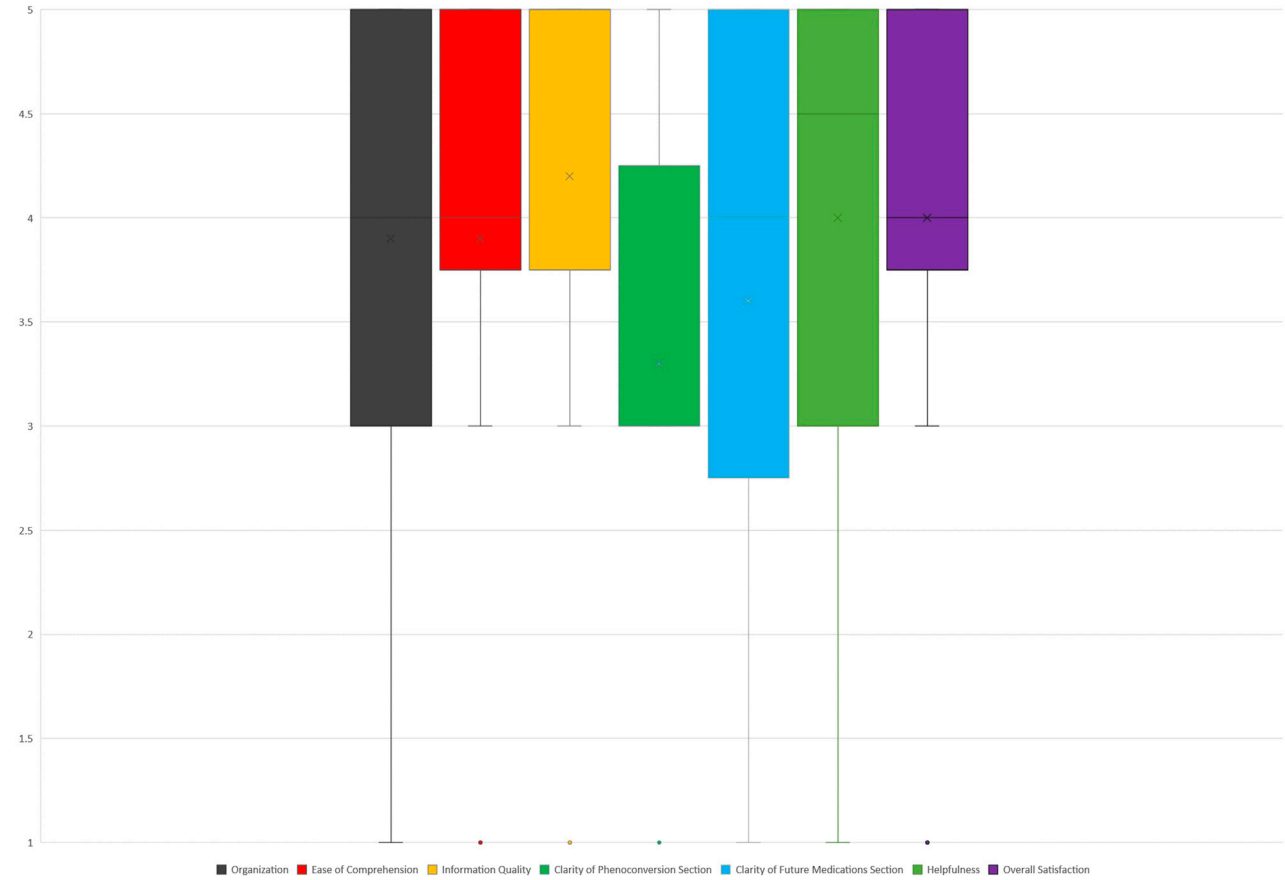

FIGURE 1
CSQU satisfaction data (1: strongly disagree, 5: strongly agree).

Table 3 presents key themes and illustrative quotes extracted from the in-depth user interviews. It highlights strengths and weaknesses identified within each section of the consult note. Figure 2 quantifies participant feedback for each consult note section. Table 4 compiles significant suggestions from participants to improve the PGx consultation note's design. Below is a summary of the main information collected for each section.

3.1 Subjective/objective section

While participants expressed overall satisfaction with the section's content, they provided valuable suggestions for enhancing the included information. Notably, in the History of Present Illness (HPI) section, Participant one suggested the importance of including the reason for the physician's test request—an idea echoed by all sessions. Additionally, some providers expressed a preference for a more detailed HPI and an extensive medication history to showcase past patient use (Participant 4).

Our note incorporates both a pre-populated list from the Electronic Health Record (EHR) and a past medication list compiled by PGx pharmacists from patient information and external medication records. Participants unanimously found the pre-populated lists unreliable and strongly favored those generated by the pharmacist, particularly since the note encompasses both current and past medications.

Regarding the inclusion of laboratory information and genotype details, most providers disagreed, citing their availability in the EHR for review (Participant 8). Alternatively, some favored the inclusion of only pertinent labs to prevent the note from becoming overly verbose (Participant 5). Finally, there was a divergence of opinions on including allergies in the note. Two providers in session three emphasized the importance of listing patient allergies in this section, while two participants in session four argued that including allergies is unnecessary due to existing chart information and proposed its removal to streamline the note.

3.2 Phenoconversion

Phenoconversion, defined as the ability of external factors, such as drug-drug-interaction, to modify a predicted phenotypic expression base on genotype, is a crucial aspect (Shah and Smith, 2015; Klomp et al., 2020; Cicali et al., 2021). Although the clarity of content in this section was acknowledged, many participants were unfamiliar with the term "phenoconversion", wf which was not explicitly mentioned in the note but surfaced during discussions. Several participants considered the information in this section

TABLE 3 Main themes and example quotes from participants.

Categories		Themes (number of participants)	Example quotes
Subjective/Objective	Strengths	General satisfaction with Subjective/Objective section. (Rosala, 2020)	"I think this is actually thorough, I do not think there's anything that is missing from the subjective section." —Participant 8 (session 5)
		EHR-generated medication list* is helpful. (Yen and Bakken, 2011)	"Yeah, I like that [EHR generated medication list] because otherwise you have to flip back to their chart. That is very pertinent in terms of interaction and influences how we're going to prescribe— knowing what else they're taking."—Participant 5 (session 3)
		*Automatically generated by EHR when medications are ordered and/or completed by various healthcare providers completing medication reconcilliations	
		List of patient's medications affected by the PGx results* (indication for the consult note) is useful. (Johnson et al., 2013)	"I think what's listed is, is appropriate in the current medications affected by the results."—Participant 7 (session 3)
		* Labeled as "Current medications affected by pharmacogenetic results:" on note	
		History of Present Illness (HPI) I section is satisfactory. (Johnson et al., 2013)	"I think this is fine."—Participant 1 (session 1)
		Important to include allergies in Subjective/Objective section. (Johnson et al., 2013)	"Do you put allergies in here or no? [moderator answers yes] Okay yeah that's an important one too."—Participant 5 (session 3)
		Pharmacist generated medication list, using outside resources and interviewing patient directly, is helpful. (Yen and Bakken, 2011)	"I loved the one you have [pharmacist generated medication list] with why they stopped them and that kind of stuff. That I would actually use and read, but not the pre-populated one."—Participant 3 (session 2)
	Weaknesses	Concern that pertinent labs (e.g., creatine, AST/ALT) may not be reliable or out of date, especially if provider looks back at note at a later date. (Johnson et al., 2013)	"I do fear that if the note is a little dated and there are more recent labs, people will References the listed labs rather than the current labs."—Participant 9 (session 6)
		List of patient's medications affected by the PGx results (indication for the consult note) is confusing. (Johnson et al., 2013)	"I found that to be confusing. I did not entirely understand if that is for newly prescribed medications that are sought for."—Participant 11 (session 7)
		* Labeled as "Current medications affected by pharmacogenetic results:" on note	
		EHR generated list of patient medications is not viewed as reliable by providers. (Yen and Bakken, 2011)	"The other list, the pre-populated one, is not reliable at all. It depends on who was taking a med history and most of the time it's completely wrong."—Participant 2 (session 2)
		EHR generated medication list provides too much information and is not necessary for the note. (Johnson et al., 2013)	"If I saw a list like this it would not be super helpful."—Participant 11 (session 7)
		Section that lists pertinent labs is unnecessary for note. (Johnson et al., 2013)	"That would be very non-meaningful. The only ones we pay attention to are creatinine since it plays a big part of post-surgical recovery."—Participant 11 (session 7)
		Including the HPI makes the note too long and this information is available elsewhere in patient chart. (Johnson et al., 2013)	"It would just make the note longer because, you know, as a treating physician, that's something that we would do anyways and probably know that from other sources."—Participant 1 (session 1)
Phenoconversion	Strength	Content within phenoconversion section* is clear and useful. (Yen and Bakken, 2011)	"It's clear, do not have anything I want to change about that section. It's good as it is, simple for us that I would not change anything there."—Participant 10 (session 7)
		* Labeled as "Relevant CYP___ Drug Interactions as of Date of note" within the note	
		Listing alternative medications to the medications affected by the PGx is helpful and relevant. (Johnson et al., 2013)	"What was helpful was listing the other alternatives that they can take."—Participant 5 (session 3)
		Phenoconversion section is important to include. (Yen and Bakken, 2011)	"I think it's the most important part because that's our guide to prescribing."—Participant 5 (session 3)
		Phenoconversion section is placed properly within the note. (Eadon et al., 2016)	"It seems to flow well where you have it."—Participant 9 (session 6)

(Continued on following page)

TABLE 3 (*Continued*) Main themes and example quotes from participants.

Categories		Themes (number of participants)	Example quotes
		Recommend keeping phenoconversion section near the top of the note. (Johnson et al., 2013)	"It should be pretty prominent near the top."—Participant 11 (session 7)
		Concern over reliability of EMR to accurately convey a patient's current medications. (Johnson et al., 2013)	"It's helpful, the trick relies on your trust and belief in the current EMR. So, the trick is to get reliable input on the EMR active medications list, and you have a static document that lives in a dynamic world."—Participant 11 (session 7)
	Weaknesses	Phenoconversion is not a well-understood concept at baseline for many providers. (Elchynski et al., 2021)	"Yeah, I would not say I'm as familiar either."—Participant 4 (session 3)
		Phenoconversion section has poor visibility for providers. (Johnson et al., 2013)	"I think I would have it stand out more and draw my attention a little more to it."—Participant 4 (session 3)
		Phenoconversion section is not important and should not be included. (Johnson et al., 2013)	"I do not like it. I do not think it's helpful."—Participant 2 (session 2)
		The phenoconversion section unclear for providers. (Johnson et al., 2013)	"I thought the sentence 'adding/replacing drugs' was a little unclear."—Participant 8 (session 5)
		Phenotype (e.g., CYP3A4) not presented in an easily digestible way and is ignored by provider. (Eadon et al., 2016)	"I kind of gloss over the phenotype to be, to be honest with you and, and maybe I should not."—Participant 4 (session3)
Assessment	Strength	Assessment section is satisfactory. (Elchynski et al., 2021)	"I love how this is done. It's really clear about whether they're controlled or uncontrolled and what considerations for the physician."—Participant 7 (session 4)
	Weaknesses	Assessment section is not helpful. (Johnson et al., 2013)	"To me seems like a slightly less helpful section of the note than some of the others, or like that test results interpretation up above."—Participant 11 (session 7)
		Assessment section should be more succinct. (Rosala, 2020)	I agree I think it's a little bit heavy and redundant for this section."—Participant 9 (session 6)
		Assessment should include all pertinent information that provider would use to make recommendation. (Johnson et al., 2013)	"For me, the assessment is a synthesis of everything that you've put together so far in this note. It's lumping in the parts of the history that were important, the parts of the labs that were important, what helped you to make the recommendation that you're going to make." —Participant 3 (session 2)
		Listing the current regimen in assessment is not relevant. (Johnson et al., 2013)	"I thought listing the current regimen is not relevant in this section because it's not part of the assessment."—Participant 8 (session 5)
Plan	Strengths	The inclusion of alternatives in the plan section is appropriate. (Johnson et al., 2013)	"I did note that you said 'switched to an alternative agent such as.' I think that wording is appropriate. I think the clinician can always adjust accordingly."—Participant 7 (session 4)
		Plan section is well received. (Yen and Bakken, 2011)	"And the plan was clear and concise."—Participant 9 (session 6)
	Weaknesses	Listing allergies is not necessary. (Johnson et al., 2013)	"I think you can remove allergies, but nothing other than that."—Participant 8 (session 5)
PGx Table	Strengths	Provider comfortable using the PGx table for future prescribing. (Johnson et al., 2013)	"I feel like when I look at this table, I'm approaching it from a perspective that this is a fixed patient response and not necessarily that it is modified by the note at the bottom."—Participant 3 (session 2)
		PGx table is useful. (Rosala)	"I think it's really interesting and helpful."—Participant 9 (session 6)
	Weaknesses	PGx table contains too much information. (Rosala)	"My experience has shown that trying to scrutinize every single interaction can be cumbersome."—Participant 2 (session 2)
		PGx table is not useful. (Johnson et al., 2013)	"So as a specialist, it's not very helpful to me, not to say it's bad, it's of less clinical use to me."—participant 11 (session 7)

(Continued on following page)

TABLE 3 (*Continued*) Main themes and example quotes from participants.

Categories		Themes (number of participants)	Example quotes
Flipped Note	Strengths	Prefers flipped note format over standard. (Longo et al., 2021)	"Yeah, I've seen this [flipped note] use more and more. Especially when I'm attending a patient, it's super helpful because often these patients are very complex and a lot of different consultants have adopted this model."—participant 4 (session 3)
	Weaknesses	Prefers standard format over flipped. (Johnson et al., 2013)	"I think it's really per person preference. I really do not care anymore. In this particular case, I think I would lean that way too [standard form], because it sort of tells a story and once someone's seen this, once they know how it tells the story and they can spend however much time they feel they need to in each section."—Participant 7 (session 4)
General	Strengths	Likes the specificity of the note template. (Johnson et al., 2013)	"We sometimes get some pharmacogenetics consult notes for studies were enrolled in and they're not this specific, they're much more general. And I wished that they were more specific like this."— Participant 7 (session 4)
		Note template is consistent with other notes seen in practice. (Eadon et al., 2016)	"The note is structured well, so it's pretty standardized."—Participant 10 (session 7)
		Overall note template is good. (Eadon et al., 2016)	"I think the template overall it's good."—Participant 5 (session 3)
		Structure of note template is good. (Eadon et al., 2016)	"I like how your notes are clear and separated into these little concise sections."— Participant 5 (session 3)
	Weaknesses	Note is too long. (Johnson et al., 2013)	"Our notes are too saturated with non-relevant information. I would condense this. This is for PGx results and it's 3 pages so it's too much."— Participant 10 (session 7)

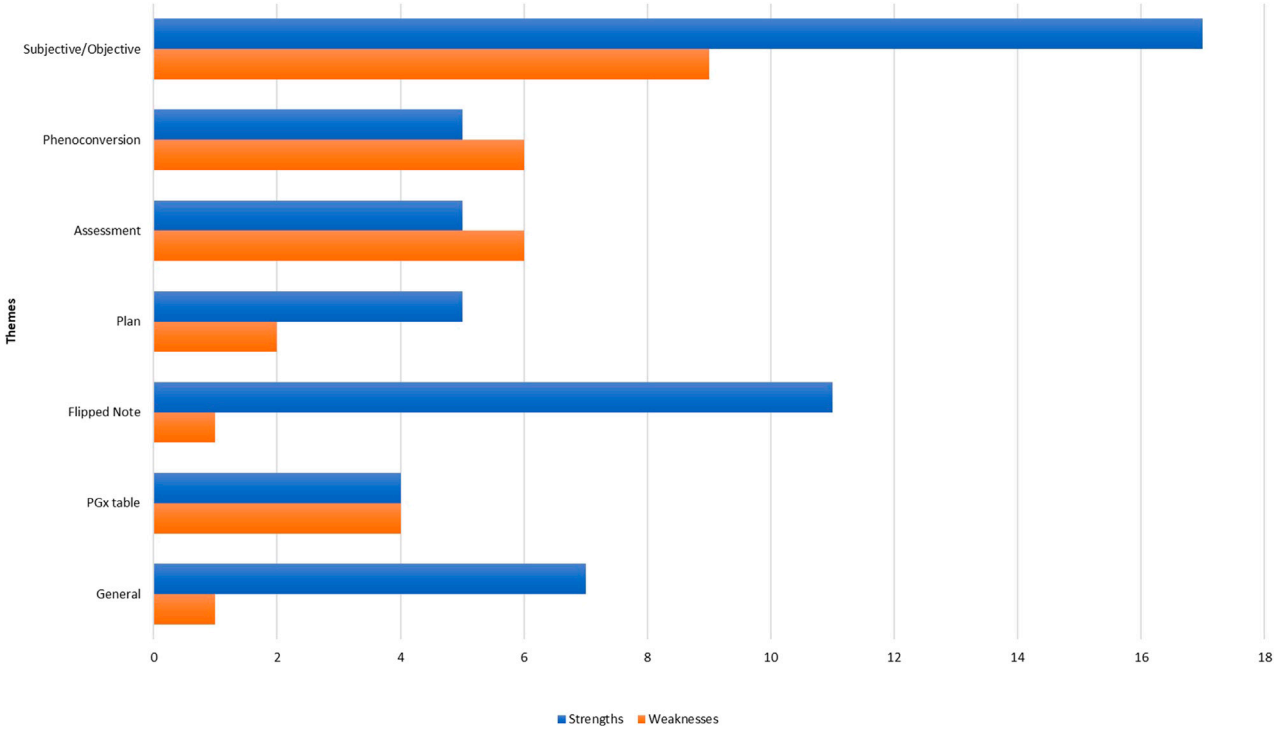

FIGURE 2
Quantifying participant comments based on type and appropriate section of the consult note.

TABLE 4 Major suggestions for improvement from participants.

Suggestion	Example quote
Include only relevant labs to medications affected by the PGx results	"I think it would have to be selective . . . it's something [labs] that would influence what we would do with medication. Because sometimes you do not want the note to get too wordy. I like being able to go in look and see if you have enough information to know what their other meds are."— Participant 5 (session 3)
Note should include list of present and past patient medications affected by PGx results	"I think it's helpful because you can see the related conditions"—Participant 7 (session 4)
Tailor the HPI to the indication for the PGx consult note. (Eadon et al., 2016)	"I think having a more niche HPI would be nice like having a more of a medication history, just to show what the patient has used in the past."—Participant 4 (session 3)
Include description of phenoconversion and recommendation. (Johnson et al., 2013)	"I think it's helpful to have the description in there, but even more important for how it will impact medication use."—Participant 7 (session 4)
Integrate note findings into EHR best practice alerts for abnormal results. (Johnson et al., 2013)	"Is there a way to integrate the phenoconversion data into the interaction checker in EPIC? Because I think that would be really neat if we could get this information within EPIC."—Participant 3 (session 2)
Assessment should include alternative medications. (Johnson et al., 2013)	"This is where it would be helpful to have suggested alternatives that would be likely to have greater safety or effect profile with the phenotype that the patient is."—Participant 11 (session 7)
Tailor PGx plan to physician specialty. (Eadon et al., 2016)	"I'd want the plan to be focused on that question [what was the consult for] but for us as a service that's looking for guidance in one domain, it would be ultimately be the most useful to have the recommendation focused on that one domain."—Participant 11 (session 7)
PGx table should have a phenoconversion column. (Johnson et al., 2013)	"I do think that table is helpful, and the additional column gives people an idea of how this applies today."— Participant 7 (session 4)
Have a uniform note label so it can be easily searched for within EPIC. (Johnson et al., 2013)	"I think just having a uniform label you can search for across encounters."—Participant 9 (session 6)
Recommended to add dispensed report (prescriptions from a variety of outpatient pharmacies)	"Is there a way you can tell which medication were dispensed? I heard you mentioning something like that, to look up what other pharmacies may be dispensed to the patient. It might be helpful to have that dispense report."—Participant 2 (session 2)

paramount, emphasizing their tendency to immediately explore recommendations for potential medication changes based on the results (Participant 4). Ten out of the eleven participants found this information beneficial, with suggestions to enhance its visibility, such as making it stand out more or potentially segregating it into its own section (Participant 4).

While some participants felt that the phenoconversion information might be better placed in the assessment section, a few did not mind its repetition in several locations of the consult note, recognizing its importance to their clinical decision-making. One participant remarked on the insufficient information in this section, and Participant five proposed that listing alternative medications would be helpful for better guidance.

3.3 Flipped note

During each user sessions, two note formats were presented to participants, and their preferences were assessed. The flipped note format was favored by the majority, with ten out of eleven participants expressing a preference for it, while one participant remained neutral. The prevailing sentiment among participants was that the flipped note format is preferable due to its ability to prominently display essential information. Participant 11 highlighted its efficiency, stating that it is "really helpful because you're cutting right to the chase," especially in complex cases involving multiple consultants.

However, there was a dissenting opinion, as one participant preferred the standard format, citing its contribution to the logical flow of the patient's story. Participant seven noted, "it is really a person preference. In this particular case, I think I would lean that way too [standard form], because it sort of tells a story." The diverse preferences underscored the subjective nature of individual preferences in note formats, with some favoring efficiency and directness while others valued a narrative structure.

3.4 Assessment section

A significant critique of the assessment section was to improve the conciseness, with providers expressing that it was "a little bit heavy and redundant for this section" (participant 9) and that it "did not feel like it is an assessment and it felt like a history instead" (Participant 3). Participants favored familiar terms such as "well controlled or poorly controlled" and advocated for brevity, suggesting that the assessment should use as few terms as possible while still effectively summarizing the information from preceding sections. Additionally, participants recommended tailoring the assessment to the specific medication that prompted the consult and including only pertinent labs for a more streamlined and relevant summary.

3.5 Plan section

While all providers acknowledged the clarity and conciseness of this section, there were varying opinions on its necessity in the note.

Some providers believed that this section was not essential, with three expressing the view that pharmacists should refrain from making clinical recommendations within the note. According to Participant 3, they were "looking for specific changes to medications" based on the pharmacogenomic (PGx) results. These providers voiced concerns about patient visibility of these notes, suggesting that PGx notes should primarily present relevant information for physicians to use in their broader clinical decision-making.

Another suggestion that emerged was the idea of tailoring the plan to the specialty of the provider who requested the note. This recommendation aimed at providing a more specialized and relevant plan, catering to the specific needs and context of the requesting healthcare professional.

3.6 PGx table

The note template concludes with a comprehensive list of other medications categorized by different indications that might be impacted by the patient's CYP polymorphism. The intention behind this table is to offer providers a reference for future use, providing insights into potential medications affected by the patient's polymorphisms. However, this table sparked the most discrepancies and differing opinions among the providers.

While seven providers found the table helpful and expressed comfort in using the information for future reference, four providers considered the table to be overly extensive and containing unnecessary details. Although most participants leaned towards a preference for a shorter table, others suggested additional information, such as including a list of alternative agents for each indication and incorporating a phenoconversion column. The diverse perspectives highlighted varying preferences regarding the level of detail and length of the table, emphasizing the need for customization to meet individual provider preferences and information needs.

3.7 General

Overall, participants expressed positive feedback regarding the PGx consultation note template's consistency and alignment with other consultation note formats. This consistency fosters familiarity and ease of use within the broader EHR system. However, participants strongly recommended reducing the note's length to improve efficiency and streamline information retrieval. Additionally, they emphasized the importance of using consistent titles for each note. This standardization would significantly enhance searchability within the EHR system, allowing clinicians to quickly locate specific PGx consultation notes and access relevant patient information.

4 Discussion

The implementation of pharmacogenomic (PGx) consultation services has become widespread across many institutions (Eadon et al., 2016; Bain et al., 2018; Longo et al., 2021). However, our study stands out as one of the first to adopt a user-centered approach for a formal assessment of provider needs and design requirements, aiming to guide future enhancements of PGx result notes. This approach allowed us to gain valuable insights into providers' workflows and preferences regarding PGx information (Andreassen and Malling, 2019). Such knowledge is instrumental for PGx specialists in tailoring consult notes to align with the clinical context, facilitating easy navigation and utilization of relevant information. Several key concepts and ideas emerged from our study, providing valuable insights for institutions looking to implement or refine PGx services (see Table 4).

Responding to requests from specialists familiar with patients who sought a shortcut to the assessment section, we also offered PGx consult notes using the flipped format, where the assessment was presented first. Concerns about redundant information in consult notes have been reported in the literature (Brown et al., 2014; Huang et al., 2018), and our study received mixed comments on note format preferences. While several participants favored the standard SOAP note for its familiarity, others preferred a more concise version using the flipped note. Therefore, we recommend providing consultations using the SOAP format but offering flip notes as an option for providers with specific requests. The SOAP note format, being more traditional, is generally easier for new providers to comprehend. Similarly, for all other sections that receive mixed comments from participants, we compile a list and discuss them with our precision medicine leadership to discuss plan for implementation and prioritization.

Our note template also introduced a new section called phenoconversion, a crucial concept in PGx consult notes that might be overlooked or not fully understood by general healthcare providers. In our study, we identified that 60% of participants did not fully comprehend this concept, indicating a need for redesign for better clarification. We propose including a short description to define phenoconversion, aiding providers in better understanding this section. Additionally, it is essential to separate and clarify the differences between current active drug-gene interactions (DGI) and potential DGIs to prevent confusion. However, capturing the current active medication list for outpatients remains challenging due to current technological limitations which only able to capture medication order information but not medication dispensing records (Lin et al., 2021).

While consultation notes traditionally serve as a direct means of consultation for requested providers, leveraging technology can make information more accessible and extend recommendations to a broader pool of providers. The ability to provide succinct information emerged as a crucial theme in our study, prompting consideration for building a "genomics profile" within patients' health record systems. The University of Florida Health has recently implemented this approach by incorporating the Epic® Genomics Module. Utilizing technology from this module, we developed language capable of explaining and providing recommendations for each drug-gene interaction relevant to a specific patient profile. This genomics profile consolidates all relevant genetic information onto a single page, facilitating easy access for healthcare providers to make optimal prescribing decisions.

Despite these insights, our study has several limitations. We collected data from a single institution, and while UF Health is a large healthcare system, the workflow and structure may not be universally applicable to other institutions. Furthermore, the use of the Epic® EHR at UF Health might not be representative of other EHR systems. Additionally, our study focused solely on physicians as the main requesters for PGx consult notes, and future research should consider collecting feedback from other healthcare providers, such as nurse practitioners or physician assistants. The use of in-depth user sessions, rather than one-on-one interview to collect feedback may lead to uneven contributions among participants, with some being more vocal than others. Lastly, our recruitment had a low response rate which might create a shwed representation of the target population.

4.1 Future directions

We plan to enhance the design of currently implemented PGx result note at our institution and disseminate a framework for other institutions who plan to implement PGx result documentation. Once we update the PGx note template, we plan to further evaluate the information provided in our PGx results note and provider satisfaction with the documentation through future provider interview. Ultimately, we will develop a practical design guideline to assist with PGx result documentation development as well as other consultation notes provided by pharmacists.

5 Conclusion

Utilizing provider feedback via in-depth user sessions and having providers complete the CSUQ regarding a PGx result note resulted in valuable feedback. The feedback collected will guide changes to the implemented PGx consult note at our institution and help create a standardized PGx consult note format.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

ND: Data curation, Formal Analysis, Visualization, Writing–original draft, Writing–review and editing. NR: Data curation, Formal Analysis, Project administration, Visualization, Writing–original draft, Writing–review and editing. BH: Data curation, Formal Analysis, Project administration, Resources, Writing–review and editing. HA: Data curation, Project administration, Writing–review and editing. LL: Data curation, Project administration, Writing–review and editing. EE: Formal Analysis, Writing–review and editing. EC: Conceptualization, Investigation, Resources, Validation, Writing–review and editing. KW: Resources, Validation, Writing–review and editing. LC: Resources, Validation, Writing–review and editing. KN: Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Validation, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2024.1377132/full#supplementary-material

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